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Full Length Research Paper

Effect of different fertilizers on the microbial activity and productivity of soil under potato cultivation

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This study was conducted to evaluate the effect of the application of different rates of mineral nitrogen, well rotten farmyard manure and *Klebsiella planticola* SL09- based microbial biofertilizer (enteroplantin) on the count of soil microorganisms (total microbial count, counts of Azotobacter, oligonitrophilic bacteria, fungi and actinomycetes), stem height and yield of potato. The experiment was set up as a randomized block design in four replications at the experimental field of the Biotechnical Faculty, Podgorica in 2008. Potato cultivar Kennebec was used as the test plant. The trial involved six treatments: non-fertilized control; N_1 treatment with 100 kg/ha CAN (calcium ammonium nitrate, 27% N); N_2 treatment with 200 kg/ha CAN; N_3 treatment with 300 kg/ha CAN; treatment with Enteroplantin– *K. planticola* SL09-based biofertilizer; and treatment with 30 t/ha solid well rotten farmyard manure. The results obtained suggested that well rotten farmyard manure induced the highest increase in microbial counts, potato yield and stem height. A similar effect on all microorganisms, except actinomycetes and fungi was seen with the use of *K. planticola* SL09-based biofertilizer. The potato yield and stem height obtained with the use of 300 kg/ha CAN was non-significantly higher than that of 200 kg/ha CAN treatment, with the count of the soil microorganisms tested been significantly reduced.

Key words: Biofertilization, microorganisms, soil, manure, mineral nitrogen, potato, yield.

INTRODUCTION

Fertilization is among the most important soil amendment operations used in modern agricultural production. Research in this area is mostly focused on increasing crop yields, with their cumulative effects (changes in soil biological and chemical properties) being often neglected. Therefore, the judicious efficient use of mineral fertilizers (nitrogenous ones in particular), organic and microbiological fertilizers can be practicable only through the adoption of a complex approach that gives importance to microbiological studies.

The count and activity of soil microorganisms as important indicators of soil biological productivity can be indicative of the economic justifiability of the use of different types, combinations and rates of fertilizers (Cerny et al., 2003; Stark et al., 2007). As a rule, microbial processes are enhanced by mineral fertilization, which is a radical method of soil nutrient balance improvement (Fauci and Dick, 1994). However, the long-term use of mineral fertilizers, particularly high rates of nitrogen fertilizers, may be harmful, as it leads to increased gaseous nitrogen losses, deteriorating physical, chemical and biological properties of the soil and, eventually, reduce safety of the plant products obtained (Barabasz et al., 2002; Ayoola and Adeniyan, 2006). It is for the these reasons that food production involving long-lasting environmental preservation and stable quality yields, is advocated within novel integrated agricultural systems. Accordingly, attention has been focused on the use of different organic substrates and biofertilizers (microbial inoculants) as an

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Table 1. Weather characteristics and long term means (LTM).

Parameter	Average air temperatures (°C) and rainfall (mm) in the research area					
	April	May	June	July	August	September
2008						
Temperature (°C)	10.10	15.70	19.90	22.10	23.00	16.00
Rainfall (mm)	123.1	75.80	105.6	17.60	39.90	102.1
LTM (2004-2008)						
Temperature (°C)	10.86	14.84	19.24	22.48	20.90	16.26
Rainfall (mm)	118.36	101.36	82.52	27.62	72.20	134.12

alternative and/or a supplement to costly mineral fertilizers. This results is improved physical, chemical and biological properties of the soil, elevated levels of readily available nutrients, phytohormones, enzymes and useful microorganisms and, hence, increased yields and safety of plant crops (Simek et al., 1999; Sofi and Wani, 2007). In this respect, the results of Emtsev et al. (1998) indicate the importance of the use of *Klebsiella planticola* TSHA-91-based bioproducts towards increased yields of vegetable crops, most notably potatoes. The authors report not only their high nitrogenase activity but also their adhesion capacity, the ability to absorb at the plant roots and colonize them throughout the growing season, and growth inhibition of some pathogenic fungi. The ability to fix nitrogen, produce biologically active matter and increase crop yield is also a characteristic of other species of the genus *Klebsiella* (Govindarajan et al., 2007; Yasmin et al., 2007; Sachdev et al., 2009).

The objective of this study was to evaluate the effect of different rates of mineral nitrogen, well rotten farmyard manure and the *K. planticola* SL09-based microbial biofertilizers, enteroplantin on soil microbial counts, stem height and yield of potato.

MATERIALS AND METHODS

The trial was set up as a randomized block design in four replications at the experimental field of the Biotechnical Faculty, Podgorica (Niksic District - latitude 42° 45' N; longitude18° 54' E; altitude 600 m), Montenegro, at the end of April 2008. Plot size was 7 m^2 (2.5 x 2.8 m) and the spacing between the blocks was 1 m. The test plant used in the experiment was potato cv. Kennebec grown on eutric cambisol [pH_{NKCl} (6.4); humus (7.65%); readily available potassium (0.185 mg/g); readily available phosphorus (0.055 mg/g); $CaCO_3$ (2.07 %)]. Planting was preceded by soil preparation and incorporation of 50 kg/ha phosphorus and 150 kg/ha calcium into the soil. The first reproduction seed material was planted into open rows at an inter-row spacing of 0.7 m and within-row spacing of 0.25 m.

The trial involved six treatments: non-fertilized control; N_1 treatment with 100 kg/ha CAN (calcium ammonium nitrate, 27% N); N_2 treatment with 200 kg/ha CAN; N_3 treatment with 300 kg/ha CAN; treatment with enteroplantin (*K. planticola* SL09-based biofertilizer); and treatment with 30 t/ha solid well rotten farmyard manure (17.4% dry matter, 13.30% organic matter, 0.37% N, 0.23% P and 0.48% K). The earlier mentioned nitrogen fertilizer and manure amounts were uniformly distributed into rows prior to potato planting. The treatment with enteroplantin involved treatment of potato tubers spaced as indicated earlier with 10 ml of the liquid biofertilizer enteroplantin. Enteroplantin is a pure culture of associative Gram-negative nitrogen-fixing *K. planticola* SL09 isolated from the tomato rhizosphere and kept in the microbial collection at the Microbiology Laboratory, Faculty of Agronomy, Cacak (Republic of Serbia). The bacterial titer in the inoculum ranged from 20 to 40 × 10^6/ml.

Standard cultural operations other than irrigation were employed during the potato growing season. Basic weather characteristics of the study area for both the growing period and five-year mean values are given in Table 1.

The soil was sampled for microbial analysis three times during the growing season (from two middle rows) at a depth of 5 to 30 cm during the following stages: I; after potato plant emergence (31 May), II; after flowering (19 June) and III; at the end of the growing season (16 August). Microbiological analyses were carried out at the Laboratory of Microbiology, Faculty of Agronomy, Cacak. They involved determination of the count of particular systematic and physiological groups of microorganisms using the method of dilution on specific solid media (Pochon and Tardieux, 1962). The following counts were determined: total microbial count (soil agar, Pochon and Tardieux, 1962), count of soil fungi (on Czapek-Dox agar, Scharlau Microbiology, 2000), count of actinomycetes (on Krasilnikov's agar, Scharlau Microbiology, 1999), counts of oligonitrophilic bacteria and Azotobacter (Fyodorov's medium, Anderson and Domasch, 1958). Microbial counts were determined by inoculation using 10^{-6} soil dilution for total microorganisms, 10^{-5} dilution for actinomycetes and fungi, 10^{-4} dilution for oligonitrophilic bacteria and 10^{-2} for Azotobacter. Incubation was followed by identification and counting of the colony forming units per 1 g of absolutely dry soil.

Potato yield was determined through the weight of tubers collected from two middle rows of each plot (expressed in t/ha). The average potato stem height (cm) was determined during the full flowering stage.

The data obtained were subjected to an analysis of variance (Statistica SPSS 5), using a two-way 6 x 3 factorial design (factor A – fertilizers used x factor B – time of sampling) for microbiological parameters and a single-factor design for stem height and potato yield. LSD test was used to test the significance of differences between individual-factor level and interaction means.

RESULTS

The results obtained suggest that soil microbiological characteristics, stem height and potato yield were significantly affected by fertilization and the growing period. The interaction between the factors analyzed, apart from

Table 2. Total microbial count (10^6/g of absolutely dry soil) in the soil under potato cultivation as dependent upon fertilization (A) and growing season (B).

A		Control	N_1	N_2	N_3	Enteroplantin	Manure	$\bar{X}_B$
	I	98.33	130.33	115.67	90.67	138.33	174.33	124.61
B	II	130.67	139.67	137.00	113.67	131.67	196.33	141.50
	III	101.67	111.67	134.67	112.00	117.33	186.00	127.22
$\bar{X}_A$		110.22	127.22	129.11	105.44	129.11	185.56	
			A		B		AB	
LSD	5%		16.21		11.46		28.08	
	1%		21.56		15.24		37.34	

Table 3. Azotobacter count (10^2/g absolutely dry soil) in the soil under potato cultivation as dependent upon fertilization (A) and growing season (B).

A		Control	N_1	N_2	N_3	Enteroplantin	Manure	$\bar{X}_B$
	I	14.33	27.67	18.00	8.00	45.33	35.00	24.72
B	II	27.00	36.67	27.33	25.00	40.00	40.33	32.72
	III	25.00	25.33	26.33	18.33	28.67	36.00	26.61
$\bar{X}_A$		22.11	29.89	23.89	17.11	38.00	37.11	
			A		B		AB	
LSD	5%		5.73		4.05		9.92	
	1%		7.62		5.39		13.19	

that in Azotobacter, was statistically non-significant.

The total count of soil microorganisms was found to be highest in solid manure treatment, which was statistically significantly higher than that in the control and other fertilization treatments. A stimulating effect was also produced by the use of enteroplantin and lower nitrogen fertilizer rates. As compared to the control, the highest nitrogen rate (300 kg/ha CAN) did not have a significant effect on the soil biological parameter tested, but induced a statistically significant decline in total microbial count as compared to the other two lower nitrogen rates applied. In terms of the growing season, the highest count of total microflora was observed during the post-flowering stage. The differences observed during the initial and final stages of vegetation were statistically non-significant (Table 2).

Solid manure, enteroplantin and the lowest nitrogen application rate had a significant stimulating effect on soil Azotobacter population. Conversely, increasing mineral nitrogen rates exhibited a decreasing tendency in the count of this physiological group of microorganisms (Table 3). The interactive effect of the fertilizers used and the growing season in this group of microorganisms showed that the highest decline in Azotobacter count, as induced by the higher nitrogen rate, was observed during the initial stages of potato development. Similar observations are applicable to the stimulating effect of the biological fertilizer enteroplantin. On the whole, the count of Azotobacter was highest in the second stage, that is, during the post-flowering stage, which was statistically significantly higher than that in the other two stages.

Oligonitrophilic bacteria, as major indicators of the soil nitrogen regime, showed high sensibility to increasing nitrogen fertilization rates. Accordingly, their count significantly decreased in the treatment with 300 kg/ha CAN. The highest count of this group of microorganisms was from the treatment with solid manure, which was comparable to the effect of the lowest rate of nitrogen fertilizer and enteroplantin (Table 4).

Solid manure showed the highest stimulating effect on soil fungal development as well. As opposed to the other groups of microorganisms, soil fungi were also highly stimulated by the three mineral fertilization rates and primarily high nitrogen rates (N_2 and N_3). Another important characteristic was the decline in their count upon enteroplantin treatment. Soil fungal count was highest in the second stage of the study, with the decline in the final vegetation stage, as compared to the previous stage, been statistically non-significant, which suggested a somewhat lower vegetation-related variability of this microbial group as compared to the other groups tested (Table 5).

As compared to the control, the actinomycete count was not significantly affected by the two lower nitrogen application rates (N_1 and N_2) and enteroplantin, but it was significantly increased by the highest nitrogen fertilizer rate used. A substantial increase was also obtained by solid manure treatment. Generally speaking, the count of

Table 4. Count of oligonitrophilic bacteria (10^5/g absolutely dry soil) in the soil under potato cultivation as dependent upon fertilization (A) and growing season (B).

A		Control	N1	N2	N3	Enteroplantin	Manure	$\overline{X}_B$
	I	19.67	24.33	25.33	14.00	21.00	28.33	22.11
B	II	28.00	39.67	30.00	23.33	35.67	36.67	32.22
	III	20.33	33.33	21.67	19.33	30.33	36.33	26.89
$\overline{X}_A$		22.67	32.44	25.67	18.89	29.00	33.78	
		A			B		AB	
LSD	5%	5.60			3.96		9.71	
	1%	7.45			5.27		12.90	

Table 5. Fungal count (10^5/g absolutely dry soil) in the soil under potato cultivation as dependent upon fertilization (A) and growing season (B).

A		Control	N_1	N_2	N_3	Enteroplantin	Manure	$\overline{X}_B$
	I	69.33	80.00	84.33	89,33	53.33	93.33	78.28
B	II	83.33	94.33	90.33	104.67	70.33	111.33	92.39
	III	80.33	84.33	93.00	103,33	59.33	98.33	86.44
$\overline{X}_A$		77.67	86.22	89.22	99.11	61.00	101.00	
		A			B		AB	
LSD	5%	9.30			6.57		16.10	
	1%	12.36			8.74		21.41	

this group of microorganisms increased until the post-flowering stage of the potato but non-significantly decreased during the final stage of the vegetation (Table 6).

Consistently with soil microbial characteristics, the highest potato yield was obtained under treatments with manure, enteroplantin and the highest mineral nitrogen rate, which was about 20% on average higher than that in the control treatment. The differences in potato yield resulting from the use of 200 kg/ha CAN were also significantly higher than that in the control. As compared to this nitrogen fertilizer rate, the yield increase under N_3 (300 kg/ha) and enteroplantin treatments was statistically non-significant. A similar observation was applicable to the average stem height of potato measured during the full flowering stage (Table 7).

DISCUSSION

Changes in soil microbial counts as induced by different types of mineral, organic and microbiological fertilizers suggest that they can be justifiably used for soil fertility and crop yield. Hole et al. (2005) reported that changes in microbial biomass serve as a parameter providing a clearer and faster response to the application of organic and mineral fertilizers to the soil, eventually affecting its potential and effective fertility. The results showed that the highest increase in microbial count resulted from the use of solid farmyard manure. Solid manure introduces a high amount of useful microorganisms and phytohormones into the soil, increases the amount of organic matter, and improves water and air relationships in the soil, thus intensifying mineralization processes and increasing the count and enzymatic activity of soil microorganisms (Mandal et al., 2007; Zhong et al., 2010). This cultural practice has a stimulating effect on the development of bacterial microflora that shows lower variation during the growing season as compared to soil fungi and actinomycetes (Balakrishnan et al., 2007; Lalfakzuala et al. 2008; Ndubuisi-Nnaji, et al., 2011). The treatment with the nitrogen-fixing biofertilizers, enteroplantin, used as a method of alternative supplementation of soil with nitrogen assimilates, induced an increase in the counts of soil bacteria, oligonitrophilic bacteria and Azotobacter (Tables 2, 3 and 4). The positive effects of biofertilizers on soil biological parameters during the first months upon treatment have been reported by other authors as well (Park et al., 2005; Biari et al., 2008; Emtsev et al., 2010). In these studies, specific importance has been given to associative biofertilizers not only in terms of regulation of soil nitrogen regime but also in terms of their role in phytohormone production, detoxication of soils contaminated with heavy metals and high salt levels, exocellular polysaccharide synthesis and other processes that result in enhanced soil biological

Table 6. Actinomycete count (10^5/g absolutely dry soil) in the soil under potato cultivation as dependent upon fertilization (A) and growing season (B).

A		Control	N_1	N_2	N_3	Enteroplantin	Manure	$\bar{X}$ B
B	I	19.00	23.00	27.33	36.00	18.00	44.33	27.94
	II	36.33	33.33	38.33	52.33	36.67	58.33	42.56
	III	31.67	35.33	28.67	49.33	35.00	46.33	37.72
$\bar{X}$ A		29.00	30.56	31.44	45.89	29.89	49.67	
		A			B		AB	
LSD	5%	7.38			5.22		12.78	
	1%	9.81			6.94		16.99	

Table 7. Potato stem height (cm) during the full flowering stage and tuber yield (t/ha) as dependent upon fertilization.

Parameter	Stem height	Tuber yield
Control	34.00^{c}	20.60^{d}
N_1	36.00^{bc}	21.26^{cd}
N_2	37.00^{ab}	22.90^{bc}
N_3	38.00^{ab}	24.13^{ab}
Enteroplantin	40.00^{a}	24.76^{ab}
Manure	37.00^{abc}	25.20^{a}

Values followed by different small letters within columns are significantly different (P<0.05) according to the LSD test.

productivity. Additionally, given the fact that some *K. planticola* strains have inhibitory effects on the development of some fungal species (Emtsev et al., 1998), the decline in the count of this group of microorganisms in the soil was an expected occurrence (Table 5). The lower mineral nitrogen fertilization rates (100 and 200 kg/ha CAN) significantly stimulated the development of the tested groups of microorganisms, which was in agreement with the results of Barabasz et al. (2002) who reported an increase in the count and diversity of bacterial, actinomycete and fungal species under lower mineral nitrogen application rates. The increased rates of mineral nitrogen (300 kg/ha CAN) led to a reduction in the count of the tested groups of microorganisms, except fungi and actinomycetes. This tendency is frequently associated with changes in the soil physical and chemical characteristics (Stark et al., 2007) as well as with the alterations in the structure of soil microbial cenosis expressed through the predominance of toxinogenic and phytopathogenic fungi. Their predominance, along with the increase in actinomycete count, can induce a decline in the count of microorganisms, particularly Gram-negative bacteria and other poorly competing species (Barabasz et al., 2002). These authors caution that inadequate nitrogen fertilization can result in the production of toxic metabolites (nitrosamines, nitro-samides, etc.) that can have not only depressing effects on most soil microorganisms but also cause teratogenic, carcinogenic and allergic effects in higher organisms (plants, animals and humans) through the food chain.

In general, the earlier mentioned microbial count dynamics during the growing season can be attributed inter alia, to the effect of climatic factors and the excretory function of the root (Bolton et al., 1992). Accordingly, the favorable soil temperature and water regimes during the post-flowering stage in potato (Table 1) induced the highest value of the soil biological parameters tested.

The effect of the applied fertilizers on the soil microbial status was quite in agreement with their effect on stem height and yield of potato (Table 7). The increase in potato yield resulting from the treatments with well rotten manure and 300 kg/ha CAN was consistent with the results obtained by other authors who reported the importance of both their individual and combined use not only in terms of yield, but also in terms of improvement of soil production characteristics and preservation of soil ecological status (Cerny et al., 2010). In this respect, Ali et al. (2009) recommended the use of an optimal rate of 70 kg/ha of pure nitrogen or an integrated system involve-ing the combined use of somewhat lower mineral fertilizer rates and farmyard manure. Manqiang et al. (2009) stressed the importance of substituting costly mineral fertilizers with manure and other organic substrates as part of the improvement of soil biological productivity and crop yield. The use of biofertilizers and their effect on potato yield and quality are given special importance in organic agriculture systems. The results obtained suggest that their use can lead to yields comparable to those obtained with 300 kg/ha CAN treatment. This effect can be associated with the pronounced ability of different species and strains of the genus *Klebsiella* to fix nitrogen and produce biologically active matter (Govindarajan et al., 2007; Sachdev et al., 2009) as well as with the growth inhibition of plant pathogenic fungi (Emtsev et al., 1998) and the reduction of stress caused by the presence of toxic substances in the soil (Pishchik et al., 2002; Emtsev et al., 2010).

Conclusion

The fertilizers used in this study had a significant effect on soil microbial characteristics, and potato growth and yield. Well rotten farmyard manure induced the highest increase in microbial count, yield and stem height of potato. A similar effect was produced by treatment with the *K. planticola* SL09-based biofertilizer (enteroplantin) in all microorganisms, except soil fungi and acino-mycetes. The yield and stem height of potato obtained upon treatment with 300 kg/ha CAN was non-significantly higher than that for 200 kg/ha CAN treatment, but the count of the soil microorganisms tested was significantly reduced.

The results obtained suggest that well rotten manure, *K. planticola* SL09-based biofertilizer and lower nitrogen rates can be successfully used in the integrated potato production system under the agroenvironmental conditions specified in this study.

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