

Research Article

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Effect of biodynamic preparations on the soil biological and agrochemical properties and coloured potato tubers quality

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Abstract: The aim of this research, which was undertaken in three years (2013–2015), was to evaluate the effect of biodynamic (BD) preparations 500 and 501 on soil biological and agrochemical properties and potato tuber quality. The soil samples were analyzed for: available phosphorus, available potassium, ammonia nitrogen, nitrogen (sum of nitrate and nitrite nitrogen) and mineral nitrogen. The potato samples were analyzed for: total polyphenols content, total anthocyanin's content and antiradical activity. The research revealed that significantly the highest concentrations of available phosphorus, available potassium, nitrogen and mineral nitrogen in the soil throughout the growing season were identified on the 14th day after soil application of BD preparation 500 (before potato planting). As well as, soil spray application of BD preparation 500 resulted in higher enzyme activity (urease and saccharase). When the BD preparation 501 was applied, the content of total phenolic compounds in the tubers of cultivars Blue Congo and Red Emmalie was significantly higher, 20.1% and 5.4%, respectively; the content of anthocyanins were significantly higher in the tubers of three tested cultivars. However, the application of this preparation did not have any effect on the content of total phenolic compounds and on the antioxidant activity of Vitelotte tubers. The use of both preparations (500+501) had significant effects

only on anthocyanins accumulation in the tubers of Blue Congo and Vitelotte. The application of preparation 500 significantly decreased the content of anthocyanins in the tubers of cvs. Red Emmalie and Vitelotte.

Keywords: Biodynamic agriculture, *Solanum tuberosum* L., nitrogen, phosphorus, anthocyanin

Abbreviations

BD = Biodynamic

TPCC = Total phenolic compounds content

DM = dry matter

1 Introduction

Over the past decade, potatoes with colored flesh (purple-fleshed and red-fleshed) have been receiving increased interest from researchers and consumers due to their richer nutritional value, antioxidant properties, appearance and flavour. They contain biologically active compounds – natural antioxidants: anthocyanins, phenolic compounds (phenolic acids, catechins, rutin), ascorbic acid, tocopherol, alfa-lipoic acid and selenium (Hamouz et al. 2010). Most of these compounds are of nutritional and pharmacological interest and determine the quality of plant raw material (Hamouz et al. 2011). The chemical composition of potato tubers with colored flesh depends on several factors such as variety, weather conditions and geographic region. Agricultural systems and cultivation technologies applied also have an impact on the biochemical composition and quality of potatoes (Nayak et al. 2011; Hejtmankova et al. 2013).

Soil and crop management practices involving intensive use of synthetic fertilizers and pesticides in the cultivation/production of plant raw materials cause long-term adverse consequences related to the safety of food products and human health (Nicolopoulou-Stamati

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et al. 2016; Mie et al. 2017). As a result, the raw materials and products produced and handled without the use of chemical agents are increasingly appreciated by the consumers (Maggio et al. 2008; Gilsenan et al. 2010).

Biodynamic agriculture is a unique system of organic agriculture, which contributes to the development of sustainable farming. The prime objective of the biodynamic agriculture involves the use of natural means to enhance soil enzyme activity (dehydrogenase) and biological diversity in a closed farming cycle, as well as produce safe food products characterised by a high nutritional value (Burkitt et al. 2007; Turinek et al. 2008; Ponzio et al. 2013). However, this system has not been comprehensively researched and applied by farmers yet.

Unlike organic farming, farmers engaged in biodynamic farming use biodynamic (BD) preparations, whose main purpose is to promote metabolism of energy and nutrients, and consequently to improve the nutritional properties of plants and soil quality (Raupp and Konig 1996, Bacchus 2010).

The globally increasing demand for the produce grown in biodynamic farms has prompted the need for scientific research aimed at multi-aspect scientific validation of the effects of BD preparations on the quality of plant raw material. There are very few data in literature describing the effects of BD preparations on the quality characteristics of potato tubers. Therefore the aim of the present study was to evaluate the effect of BD preparations 500 and 501 on soil biological and agrochemical properties and potato tuber quality.

2 Materials and methods

2.1 Plant material

The study was conducted using the three cultivars of potato (*Solanum tuberosum* L.) with colored flesh (Blue Congo, Vitelotte – purple flesh and Red Emmalie – red flesh) and biodynamic (BD) preparations used in their cultivation technology.

2.2 Field experiment

The field experiment was conducted in 2013–2015 on an organic farm in Prienai district (Lithuania). Potatoes were planted in May, and harvested in September. They were fertilised with plant compost (30 t ha⁻¹). The chemical composition of plant compost was as follows: concentrations of nitrogen 4.3 g kg⁻¹, available phosphorus

1068.21 mg kg⁻¹, available potassium 801.6 mg kg⁻¹ and pH_{KCl} 6.69. Weeds were controlled by mechanical measures. The experimental plots were arranged in a randomised design with four replications. The total plot size was 175 m² (5 × 3.5 m) and the size of a harvested plot was 10 m² (4 × 2.5 m), the width of the protection zone was 0.5 m.

2.3 Experimental design

Four experimental treatments were implemented to estimate the effects of the BD preparations. A two-factor experiment was set up: I – three coloured potato cultivars (Red Emmalie, Vitelotte, Blue Congo), II – treatment with BD preparations as field sprays (four treatments): 1 – preparations not used – control treatment; 2 – the soil was sprayed with preparation 500; 3 – potato plants were sprayed twice with preparation 501; 4 – the soil was sprayed with preparation 500, potato plants were sprayed twice with preparation 501. The BD preparations 500 and 501 were purchased from the Demeter certified farm (CvW KG, Internationale Biodynamische Präparatezentrale, Germany). BD preparation 500 is fermented manure. Its composition: available phosphorus (P₂O₅) – 1751.96 mg kg⁻¹, available potassium (K₂O) – 259.20 mg kg⁻¹, nitrogen – 2.10%, pH – 6.96, urease enzyme activity – 1.56 mg NH₃ 1 g soil per 24 h, saccharase enzyme activity – 32.7 mg glucose 1 g soil per 48 h. BD preparation 501 is quartz crystal rock, ground very finely.

2.4 Soil preparation and analyses

Soil samples for the determination of the general agrochemical characteristics were taken before the establishment of the trial. To estimate the effects of BD preparations, the soil was sampled 5 times – before the spray application and 7 days, 14 days (before potato planting), 63 days (during potato organogenesis stages VIII–IX) and 126 days (shortly before harvesting) after the spray application with BD preparation 500. In each replication of the treatment, composite soil samples were taken from 5 different places from the arable layer (0–20 cm depth) using a Nekrasov auger. The samples were air-dried, crushed in a porcelain mortar and sieved with a 2 mm sieve. The soil samples were analysed for: available phosphorus (P₂O₅) concentration mg kg⁻¹ by the CAL method using a spectrophotometer Beckman DU-40; available potassium (K₂O) concentration mg kg⁻¹ by the CAL method using a flame photometer Corning PC-410; ammonia nitrogen and nitrogen (sum of nitrate and

nitrite nitrogen) concentrations mg kg^{-1} by a flow injection analysis (FIA) spectrometric method using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Finnigan MAT, Germany); mineral nitrogen concentration mg kg^{-1} was calculated as a sum of nitrogen (nitrate and nitrite) and ammonia nitrogen; activity of soil enzymes – urease was measured after Hofmann and Schmidt (1953) methods and saccharase – after Hofmann and Seegerer (1950) methods, 4 times - before the spray application and 7 days, 14 days and 126 days after the spray application with BD preparation 500.

2.5 Potato tuber quality analyses

For tuber quality laboratory analyses, 5 kg samples were randomly collected from each plot of a treatment of which a composite 20 kg sample was formed. Potatoes were washed with tap water, dried with the use of paper towels and cut into slices of approximately 10 mm. Slices of raw tubers were frozen at -20°C . The samples were freeze-dried using a lyophilizer (BOC Ltd., Edwards, England, type 5411). Dried slices were ground using an electric grinder (GmbH Retsch, Germany, type GM 200) and packed in plastic boxes and stored under refrigeration at 4°C until further investigation.

The potato samples were analyzed for: total phenolic compounds ($\text{mg } 100 \text{ g}^{-1}$ dry matter) by the Folin-Ciocalteu colorimetric method (Gao et al. 2000); total anthocyanins ($\text{mg } 100 \text{ g}^{-1}$ dry matter) by the high-performance liquid chromatography (HPLC) (DIONEX Ultimate 3000, Germering, Germany with a PDA detector, a Cadenza Imtakt C5-C18 (75 x 4.6 mm, $5\mu\text{m}$) column with a guard column was used; mobile phase solution: A – 4.5% formic acid, B – acetonitrile, eluent flow-rate 1 ml per min; gradient: 0-1 min 5% (B), 20 min 25% (B), 21 min 100% (B), 26 min 100% (B), 27 min 5% (B)). Column temperature were 30°C ; antiradical activity $\mu\text{mol TE g}^{-1}$ – by the ABTS radical-cation scavenging method. Antiradical activity of potato extracts were expressed as the trolox equivalent antioxidant capacity (TEAC). Measurement of the antiradical activity was carried out according to Re et al. (1999). The results were expressed in trolox equivalents $\mu\text{mol l g dry matter (DM)}$.

2.6 Statistical analysis

All analyses were replicated four times. The data were statistically processed using the analysis of variance (ANOVA) method from the software package STATISTICA

(Statistica 7; StatSoft, Inc., Tulsa, OK, USA). Arithmetical averages of the experimental data were calculated. The statistical significance of differences between the means was estimated by Fisher's LSD test ($P < 0.05$).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Soil quality

Soil fertility is determined by the optimal regime of the major nutrients, such as nitrogen, phosphorus and potassium. When these elements are in short supply in the soil, crops under-perform and yields suffer (Deng et al. 2014). Our research findings suggest that significantly the highest concentrations of available phosphorus, available potassium, nitrogen (sum of nitrate and nitrite nitrogen) and mineral nitrogen in the soil throughout the entire growing season were identified on the 14th day after soil application of preparation 500 (before potato planting) (Table 1). From the potato planting until the end of the growing season the contents on nutrients in the soil were decreasing because of the uptake by the plants.

It was found that 14 days after the soil application of preparation 500, the soil contained significantly higher contents of major nutrients: available phosphorus increased by 9.37%, available potassium by 5.66%, nitrogen (sum of nitrate and nitrite nitrogen) by 22.72%, and mineral nitrogen by 16.74% (Table 1). Significantly higher concentrations of available phosphorus, nitrogen (sum of nitrate and nitrite nitrogen) and mineral nitrogen were also established in this treatment 63 and 126 days after application compared with the control treatment (unsprayed soil).

It may be presumed that preparation 500 activates soil microbiological processes during which intensive organic matter mineralization and humification occur. In this way, microorganisms supply plants with nutrients by processing the relatively stable organic compounds into the form which is readily available for plants. Researchers have indicated that sporic bacteria species attributable to *Bacillus* genus predominate in the BD preparation 500 (Giannattasio et al. 2013), which decompose complex organic compounds in the soil.

One of the most important and most accurate soil biological activity indicators is soil enzyme activity. They are the mediators and catalysts of important soil functions that include: decomposition of organic inputs;

Table 1: The effect of BD preparation 500 on soil agrochemical indicators

Soil agrochemical indicators	Not sprayed	Sprayed with P500		Not sprayed	Sprayed with P 500	Not sprayed	Sprayed with P 500
	Days after spray application						
	Before spray application	7 days	14 days	63 days		126 days	
Available phosphorus, mg kg ⁻¹	156.52Bb	160.40Bb	171.19Aa	112.64Db	129.78Ca	103.20Eb	112.83Da
Available potassium, mg kg ⁻¹	217.32Bb	222.47Bb	229.62Aa	88.21Ca	91.90Ca	79.14Da	81.01Da
Nitrogen (sum of nitrate and nitrite nitrogen), mg kg ⁻¹	13.51Bb	13.38Bb	16.58Aa	8.81Db	10.09Ca	7.36Eb	8.40Da
Ammonia nitrogen, mg kg ⁻¹	3.50Ba	3.30Ba	3.30Ba	4.62Aa	4.59Aa	4.06Aa	4.14Aa
Mineral nitrogen, mg kg ⁻¹	17.03Bb	16.74Bb	19.88Aa	13.37Db	14.71Ca	12.44Eb	13.90Da

Note: *factor A* – spray application time: before spray application, 7, 14, 63 and 126 days after spray application; *factor B* – spray application treatment: not sprayed, sprayed with preparation 500 (P 500). The differences between the means of treatments of *factor A* marked by not the same letter (A, B, C, D, E) and between the means of treatments of *factor B* marked by not the same letter (a, b) are significant at $P < 0.05$.

transformation of native soil organic matter; release of inorganic nutrients for plant growth (Dick 1997). Our study showed that soil spray application of preparation 500 resulted in higher enzyme activity (Table 2). Seven days after the spray application, the activity of the soil enzymes urease and saccharase insignificantly increased. Significantly higher activity of the tested soil enzymes were recorded 14 and 126 days after the spray application: urease activity increased by 25.00% and 40.54% respectively and that of saccharase by 6.74% and 15.74%, respectively. Researchers maintain that saccharase activity indicates intensification of organic matter mineralization processes in the soil and urease activity is related to the humification processes occurring in the soil (Baležentienė 2014). Other researchers also found that in soil where BD preparation 500 had been applied, the soil enzymes activity in the soil were higher (Carpenter-Boggs et al. 2000; Zaller and Köpke 2004; Juknevičienė 2015).

3.2 Potato tubers quality

The findings of the current research suggest that the total phenolic compounds content (TPCC) in the tubers of the tested cultivars varied from 248.62 to 350.40 mg 100 g⁻¹ DM (Table 3). The TPCC in the tubers of cv. Vitelotte was significantly 23.43% higher than that in the tubers of cv. Blue Congo, and as much as 36.60% higher than that in the tubers of cv. Red Emmalie.

The application of preparation 501 sprayed on the potato plants had significant effects on the accumulation of TPPC only in tubers of cvs. Red Emmalie and Blue Congo cultivars (Table 3). In the tubers of Red Emmalie

the TPCC increased by 5.41% and that in the tubers of Blue Congo by 20.13% compared with the control treatments. Such results can be explained by the fact that the plants assimilate silicon dioxide, which can affect changes in light wavelength and/or light interception in plants (Bacchus 2010), which determines synthesis and accumulation of secondary metabolites in tissue. However, the use of preparation 501 did not have any effect on the TPCC in the tubers of cv. Vitelotte. The scientific literature indicates that individual plant species and even varieties have different mechanisms of silicon absorption (Sahebi et al. 2015). Compared with the control treatment, the preparation 500 showed a trend towards reducing the TPCC in all potato cultivars studied; however, a significant reduction was observed only for tubers of cv. Vitelotte (Table 3). The use of both preparations together (500+501) did not have a significant effect on the TPCC in the tubers of the tested cultivars.

Researchers' opinions on how BD preparations affect the accumulation of phenolic compounds in plants differ. Research has shown that lettuce (Heimler et al. 2012) and mangos (Maciel et al. 2011) sprayed with BD preparations resulted in higher contents of phenolic compounds, compared with the ones grown using chemicals. However, Germany and Switzerland scientists compared the TPCC in wheat grown in biodynamic, ecologic and intensive systems, however, did not establish any significant differences (Langenkamper et al. 2006).

Anthocyanins are members of the flavonoid group of phytochemicals, which are present in various parts of plants. The amount of these compounds in potato tubers may depend on a host of factors, cultivar being one of the key ones (Lachman et al. 2012). Significantly the

Table 2: The effect of BD preparation 500 on soil enzyme activity

	Not sprayed	Sprayed with P 500		Not sprayed	Sprayed with P 500
Soil enzymes	Days after spray application				
	Before spray application	7 days	14 days	126 days	
Urease activity, mg NH ₃ 1 g soil per 24 h	0.36Cb	0.37Cb	0.45Ba	0.37Cb	0.52Aa
Saccharase activity, mg glucose 1 g soil per 48 h	37.21Bb	37.59Bb	39.72Aa	32.60Cb	37.73Ba

Note: *factor A* – spray application time: before spray application, 7, 14, 63 and 126 days after spray application; *factor B* – spray application treatment: not sprayed, sprayed with preparation 500 (P 500). The differences between the means of treatments of *factor A* marked by not the same letter (A, B, C, D, E) and between the means of treatments of *factor B* marked by not the same letter (a, b) are significant at $P < 0.05$.

Table 3: The effect of BD preparations 500 and 501 on the total phenolic compounds content (TPCC), total anthocyanins content and antiradical activity in potato tubers with colored flesh

Potato cultivar Not sprayed	Spray application treatments			
	P 500*	P 501	P 500*+501	
	Total phenolic compounds content, mg 100 g ⁻¹ DM			
‘Red Emmalie’	256.51Cb	248.62Cb	270.39Ba	268.62Cab
‘Blue Congo’	283.89Bb	272.70Bb	341.04Aa	282.60Bb
‘Vitelotte’	350.40Aa	317.42Ab	341.68Aa	333.63Aa
	Total anthocyanins content, mg 100 g ⁻¹ DM			
‘Red Emmalie’	157.51Bb	134.40Bc	174.04Ba	153.60Bb
‘Blue Congo’	58.19Cc	60.20Cc	93.01Ca	70.20Cb
‘Vitelotte’	266.58Ab	186.56Ac	278.78Aa	282.10Aa
	ABTS radical-cation scavenging method, μmol TE g ⁻¹ DM			
‘Red Emmalie’	13.60Cb	13.12Cb	15.78Ca	14.25Cb
‘Blue Congo’	16.46Bb	17.29Bb	17.59Ba	16.32Bb
‘Vitelotte’	24.72Aa	18.98Ab	24.47Aa	23.90Aa

Note: * preparation 500 (P 500) applied on the soil, preparation 501 (P 501) applied on plants. Different capital letters (A, B, C) in the same column and different small letters (a, b, c) in the same row indicate significant differences between cultivars (*factor A*) and spray application treatments (*factor B*) at $P < 0.05$.

highest anthocyanins content (266.58 mg 100 g⁻¹ DM) was accumulated by the cv. Vitelotte, while the lowest content (58.19 mg 100 g⁻¹ DM) was recorded for the cv. Blue Congo (Table 3). Significant differences in the contents of this pigment were identified between the tested cultivars.

Our research findings suggest that the greatest positive effect on the content of anthocyanins in potato tubers was exerted by the preparation 501 (Table 3). It gave a significant increase (4.58 to 59.84%) in the content of anthocyanins in the tubers of all tested cultivars. Under the effect of the preparations 500+501, the content of anthocyanins significantly increased by 20.64% in the tubers of cv. Blue Congo and by 5.82% in the tubers of cv. Vitelotte compared with the control treatment.

However, in the treatment where the soil had been spray applied only with the preparation 500, significantly lower anthocyanins content was determined in the tubers of cvs. Red Emmalie and Vitelotte. Heimler et al. (2012) found a higher content of anthocyanins in Batavia lettuces sprayed with BD preparations. However, research on grapes by Reeve et al. (2005) showed that contents of anthocyanins and phenolic compounds were significantly higher in grapes sprayed with BD preparations in one year only.

The ABTS radical-cation scavenging method was used to estimate the antiradical activity of the bioactive compounds present in potato tubers. The highest antiradical activity was identified for the tubers of Vitelotte (24.72 μmol TE g⁻¹ DM), and the lowest - for the tubers of

Red Emmalie (13.60 $\mu\text{mol TE g}^{-1}\text{ DM}$) (Table 3). Significant differences in the antiradical activity were established among all the cultivars tested. Higher antiradical activity of Vitelotte tubers, compared with the other cultivars tested can be associated with higher phenolic compounds content in them as well as with the qualitative composition of these compounds.

The findings of the present study suggest that preparation 501 significantly increased the antiradical activity of Red Emmalie and Blue Congo tubers by 1.16 and 1.07 times, respectively, whereas soil application of the preparation 500 reduced the antiradical activity of tubers of all cultivars tested; however, this reduction was significant only in the tubers of Vitelotte (Table 3). The use of both preparations together (500+501) did not have a significant effect on the antiradical activity in the tubers of the all tested cultivars.

Higher antioxidant activity was identified in apples (Carbonaro et al. 2000), mangos (Maciel et al. 2011) and Batavia lettuce (Heimler et al. 2012), which were grown using BD preparations. However, Tassoni et al. (2014) compared the antioxidant activity between white (Albana) and red (Lambrusco) grapes and the wine made from them, grown on biodynamic, ecologic and intensive farms, and did not determine significant differences between the different farming systems.

4 Conclusions

Our results indicated that the use of the preparation 500 stimulated the activity of soil enzymes saccharase and urease and increased the contents of nutrients important for plant growth (available phosphorus, nitrogen (sum of nitrate and nitrite nitrogen) and mineral nitrogen) in the soil.

According to the chemical composition, the tubers of cv. Vitelotte were found to be the most valuable: they accumulated significantly the highest contents of phenolic compounds, anthocyanins and were characterised by the highest antiradical activity.

The application of preparation 501 sprayed on the potato plants had positive effects on the total accumulation of anthocyanins, TPPC as well as antioxidant activity in tubers of Blue Congo and Red Emmalie cultivars. However, in Vitelotte tubers this preparation had no significant effect on the TPPC and on the antioxidant activity. When the combination of both preparations (500+501) were applied, positive effects were shown only for the accumulation of anthocyanins in tubers of Blue Congo and Vitelotte. Application of preparation 500 significantly

reduced the content of anthocyanins in the tubers of cvs. Red Emmalie and Vitelotte and the TPPC in the tubers of Vitelotte.

Conflict of interest: Authors declare no conflict of interest.

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