



RESEARCH ARTICLE

IMPACT OF FERTILIZER PRODUCTS OF "AGROPOLYCHIM" AD ON CHEMICAL INDICATORS AND ENZYMATIC ACTIVITY OF SOIL IN COMMON WINTER WHEAT AND OILSEED RAPE

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ABSTRACT

The scope of current research is to establish the impact of different fertilizers (ammonium nitrate, mono- and diammonium phosphate, liquid nitric fertilizer, urea and compound fertilizer NPK) produced by "Agropolychim" AD on chemical parameters and soil enzymatic activity related to common winter wheat and oilseed rape. Fertilizers are applied before sowing process. The experiment is repeated twice into greenery. Biogenic elements and enzymatic activity estimation is made after soil sampling and during wheat pick up phase and at the end of coleseed's leaf rosette phase. Results show that the highest total nitrogen volumes in wheat samples are detected when fertilization with DAP is made, and in the cases with coleseed – the highest total nitrogen content is estimated when liquid nitrogen fertilizer UAN is applied. Total phosphorous content in both cases is highest when fertilization with DAP is done. Recorded content of total potassium in wheat and coleseed samples is highest in cases when compound fertilizer NPK is applied. It has been established higher content of these three macronutrient elements in case of wheat experiment. This fact proves coleseed's larger needs of nutrient elements even in the early phase of culture development. Used fertilizers enhance the enzyme soil activity. The values for catalase and cellulase activity are higher with fertilized soils samples compared with control base case - soil sample without fertigation. Application of Ammonium nitrate and NPK increases catalase activity. In case of DAP application, in contrary, the activity of this enzyme is decreased as well as with wheat and coleseed. It has been estimated that cellulase activity is around 19% higher when fertilizers are applied compared with base case of non-treated soil with fertilizers. The best result for cellulase activity value is achieved with UAN application in both cases – wheat and coleseed. Tested chemical and enzymatic parameters can be used as specific chemical and biochemical markers for estimation of fertilized soils.

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INTRODUCTION

Wheat and coleseed are base winter crops in Bulgaria. For 2013 the areas are 1,20 mil ha and 0,13 mil ha respectively (<http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#anchor>). Due to the fact that the above mentioned crops are fast/intensive growing, it is necessary to examine the optimal fertilization levels to allow good crops progress on the one hand and on the other to not lead to soil deterioration in a long term period. Nutrients export via 1 t production as an active substance is: 20 kg/ha N, 7 kg/ha P₂O₅, 6 kg/ha K₂O (in wheat) and 32 kg/ha N, 16 kg/ha P₂O₅, 8 kg/ha K₂O (in coleseed) (Nikolova et al., 2014).

Thus, the coleseed extracts more macronutrients from the soil, fact that should be bear in mind when optimal levels for fertilization are determined. Soil fertilization reflects differently on the enzymatic activity and soil fertility. Martens et al. (1992) reports for a long term study demonstrating that applying of the organic matter maintains high levels of soil phosphatase. Long term phosphoric fertilization increases phosphorous content above plants need (De Laune et al., 2006). Fertilization process increases the activity of all enzymes taking part into the organic base oxidation processes, as well as in hydrolysis of the cellulose, hemicellulose, proteins and urea as a result of increased microbial biomass and higher availability of substrate (Giacometti, 2013). According Zhang et al. (2015), applying of urea and combined urea contained product for a period more than 20 years plus horse manure increases crops yield respectively by 31% and

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69%. On the other hand, fertilization only with high volumes of urea leads to soil acidification and salinization as well as decreasing of total organic carbon content. The same authors establish that fertilization with urea increases the activity of enzymes α -galactosidase and β -galactosidase while decreases the activity of α -glucosidase and β -glucosidase. The conclusion is that inorganic fertilization affects differently on enzymatic carbon transformation. Urea and horse manure applying increases the enzyme activity and content of total nitrogen, water soluble organic carbon and microbial biomass. Skwaryło-Bednarz and Krzepińko (2013) found that plants catalase activity increases as a result of increased NPK content in soils via fertilization process (leaves and seeds). The catalase is one of the most important enzymes involved in regulating the processes of plants growth and development (Mioduszevska, 2001).

This enzyme reacts extremely fast with hydrogen peroxide compared with typical plant peroxidases (Bartos, 2009). Catalase level is a specific marker during plant physiological development (Szymczak *et al.*, 2011). Chemical fertilization with phosphorous and nitrogen contained fertilizers for a period more than 17 years in soils with soybean, sorghum and millet (Qiang *et al.*, 2013) reports that chemical indicators are changed significantly more than soil physical properties. Quality indicators of soil top layer are more variable and sensitive compared with the lower soil layers. It is necessary to know the impact of different fertilization norms and combination between nutrients on the overall biological soil activity - prerequisite for effective and reasonable application of mineral fertilizers and protection of the environment from pollution (Bogdanov and Ilinkin, 2013). Many researchers have studied the influence of fertilization on the soil fertility by exploring soil enzymatic activity (Jia *et al.*, 2001; Liu, 2004). These studies, however, are namely focused on a static effect of fertilization on soil microorganisms and their enzyme activity. Very few studies have been conducted for evaluation of fertilization influence on the dynamic changes of soil enzyme activities. Although several studies are done with regards influence of long term chemical fertilization on soil parameters including physical properties of the soil, chemical and biological indicators, they are focused on a soil structure (Masto *et al.*, 2006; Celik *et al.*, 2010), soil humidity (Song *et al.*, 2010), content of organic carbon and its distribution (Tarinder *et al.*, 2008), microbial biomass (Périé and Munson, 2000; Giacometti *et al.*, 2013), soil enzyme activities (Liu *et al.*, 2010; Karaca *et al.*, 2011) and other parameters. All these factors for determination of soil quality during fertilization process have to be considered integrated in order to define soil potential in terms of its fertility as well as to reduce its pollution (Edmeades 2003; Romaniuk *et al.*, 2011.; Yan *et al.*, 2011). Soil enzymes enable integrated biological estimation of soil functions (Nannipieri *et al.*, 2002). They play a key biochemical functions during the overall transforming process of contained in the soil organic substances (Burns, 1978, 1983). These sensitive indicators may serve as specific biochemical markers for determining the condition of the soil. The aim of current scientific study is to follow up the influence of various mineral fertilizers produced by "Agropolychim" AD on some chemical parameters and soil enzymes. Studied are two types of crop: winter common wheat and oilseed rape.

MATERIALS AND METHODS

The experiment was initiated in autumn 2014 year in uncontrolled polycarbonate greenhouse owned by Technical

University, Varna in areas with dimensions 30x30 cm. Two repetitions are made. Winter common wheat and oilseed rape have been fertilized as follows with:

- Ammonium nitrate (NH_4NO_3);
- Diammonium phosphate (Diammophos, DAP);
- Monoammonium phosphate (Ammophos, MAP);
- Liquid nitrogen fertilizer (UAN);
- Urea;
- Complex fertilizer (NPK).

Separate test is made for each crop without applying of fertilizers, so called control variant. Fertilizer products are applied before sowing period with specific consumption (fertilization norm) 5 kg active substance (with wheat) and 3 kg active substance (with coleseed) calculated depending on the volume of each repetition. In Table 1 is presented content of active substance as mass % in used mineral fertilizers (<http://agropolychim.bg/>). Agrochemical soil analyze is done for determination of nitrogen, phosphorous and potassium before applying the fertilizers. Ammonium nitrogen ($\text{NH}_4 - \text{N}$) content is determined by means of photometry, using indophenol blue as a result from extraction process with calcium dichloride solution (CaCl_2). Nitrate nitrogen ($\text{NO}_3 - \text{N}$) is determined by means of photometry, using Nitrospectral as a result from extraction process with calcium dichloride solution (CaCl_2). Content of phosphorous and potassium is determined by means of "double" lactate method developed by Egner-Riehm. This method is based on the extraction of "movable" phosphorous and potassium compounds with calcium lactate solution $(\text{CH}_3\text{CH.OH.COO})_2\text{Ca}$ that is treated with buffer solution of hydrochloric acid up to pH 3.5-3.7 at a ratio soil : solvent = 1:50 and time period for reaction around 90 minutes.

Analyzes of examined soil biogenic nutrients for each of the variants are carried out in the autumn of 2014 at the beginning of the experiment, as well as in February 2015 during tillering stage of wheat and at the end of rosette stage of coleseed. All soil analyzes has been conducted in Laboratory of soil science in Technical University, Varna. Obtained agrochemical data were statistically processed and results are included in a dispersion analyze by calculating the smallest evidenced difference between separate variants at $p = 0.05$ ($\text{LSD}_{0.05}$). The values for variation coefficients (VC) are estimated. Statistical processing is done by means of software product STATISTICA, version 10. Cellulase activity is tested by means of laboratory analyze (Khaziev, 1976). In a Petri dish a sample of soil with around 7 mm depth is put meanwhile keeping 60% marginal field moisture capacity. Three sterile filter paper strips with dimensions 10/50 mm are put above the soil layer, following by a process of tempering in a thermostat at 25 °C. In a period of 15 days a percentage of degradation is estimated using grid-pattern. Soil catalase activity is determined via metrics analyses with manganese (Khaziev, 1976). Statistical data processing of the enzymatic activity includes calculation of the average value from three repetitions and standard deviation.

RESULTS AND DISCUSSION

In Table 2 are presented results from soil samples test before applying of the fertilizers. Comparing the achieved results with statistical data for measured content of biogenic elements in arable lands in Bulgaria for 2005-2011, presented by the

Executive Environmental Agency, it is determined that nitrogen availability in the soil is above average value for country (1,66 g/kg), and phosphorous availability is good but still below established farmland's average levels (845,65 mg/kg) (<https://eea.government>). The soil reaction (pH value) in current experiment is slightly acidic (pH=6.5) and it is suitable for crops development. In Table 3 are presented the results from soil analyze for content of total nitrogen, phosphorous and potassium with wheat. It has been found that total nitrogen values in samples fertilized with ammonium nitrate, DAP, MAP and UAN are in a relatively narrow range: 3,46 g/kg using DAP and 3,18 g/kg applying MAP. Comparing with the above mentioned, the cases with urea and NPK applying have lower value for total nitrogen content relatively with 35%. No statistically proven difference between achieved values for total nitrogen content using only these fertilizer products.

content is estimated in cases of MAP (581,43 mg/kg) and DAP (571,51 mg/kg) fertilization, but difference between these results is not proved. The lowest data are read in case of ammonium nitrate (501,96 mg/kg) and UAN (484,01 mg/kg) application. Evaluating all above mentioned data, the conclusion is that wheat assimilates macronutrient elements during initial stages of growing. Well known fact is that tillering of wheat begins in autumn when an optimal fertilization before sowing is made followed by high quality sowing process and good water availability; 2-3 tillers are developed. Tillering continues in spring but the autumn tillers are more productive compared with spring ones. That is why the aim is all necessary nutrient elements for crop to be available at very beginning of the process (<http://www.naas.government.bg/bg/download?id=2196>). As a result of fertilizers application no changes in soil reaction (pH) are observed.

Table 1. Mineral fertilizers, produced by „Agropolychim“ AD, used in a research

Fertilizers	Active substance
Ammonium nitrate (AN)	34,5% N
Liquid nitric fertilizer (UAN)	32% N
Urea	46% N
Monoammonium phosphate (MAP)	11% N, 52% P ₂ O ₅
Diammonium phosphate (DAP)	18% N, 46% P ₂ O ₅
Complex fertilizer (NPK)	14% N:14% P ₂ O ₅ :14 % K ₂ O+11,5% S

Table 2. Content of total nitrogen, phosphorous and potassium before fertilization

sample	pH	N, g/kg	P, mg/kg	K, mg/kg
soil	6,5	2,53	721,8	598

Table 3. Chemical parameters for tested soil samples with wheat

Crop	Fertilizer types	pH	N, g/kg		P, mg/kg		K, mg/kg	
			Aver.	VC	Aver.	VC	Aver.	VC
Wheat	NH ₄ NO ₃	6,95	3,39	0,54	700,39	0,08	501,96	0,83
	DAP	6,96	3,46	1,43	916,81	0,09	571,51	1,66
	MAP	6,63	3,18	0	885,19	0,06	581,43	0,49
	UAN	6,84	3,29	0,52	701,65	0,09	484,01	3,76
	Urea	6,95	2,65	0,37	720,65	0,56	547,39	1,73
	NPK	6,71	2,64	1,45	799,66	0,93	659,82	0
	Control case	6,98	2,12	0,30	674,85	0,07	483,61	3,92
LSD _{0,05}			0,07		19,01		34,04	

Table 4. Chemical parameters of soil samples with coleseed

Crop	Fertilizer types	pH	N, g/kg		P, mg/kg		K, mg/kg	
			Aver.	VC	Aver.	VC	Aver.	VC
Coleseed	NH ₄ NO ₃	6,89	2,08	0,17	688,51	0,08	455,73	0,13
	DAP	6,41	2,12	0,53	910,34	0,03	453,86	1,88
	MAP	6,91	2,11	0,13	883,54	0,03	479,72	1,58
	UAN	6,89	2,18	0,42	697,69	0,04	459,35	0,21
	Urea	6,83	2,14	0,50	711,55	0,07	552,75	0
	NPK	6,69	2,16	0,29	871,42	0,06	572,72	1,59
	Control case	7	1,90	0,11	660,73	0,07	419,82	0,41
LSD _{0,05}			0,02		249,61		20,37	

As for the obtained results for total phosphorous content, clearly stand variants with DAP (916,81 mg/kg) and MAP (885,19 mg/kg) fertilization followed by NPK (799,66 mg/kg). In case of urea application the nutrient value is lower. The smallest phosphorous content is estimated in cases of UAN (701,65 mg/kg) and ammonium nitrate (700,39 mg/kg) fertilization, as no statistically proven difference only between these results. Observing nutrient element potassium – the highest and reliable content in wheat sample is estimated in case of NPK application (659,82 mg/kg) as not reported variation between the two replications. Around 20% lower

With all samples the reaction is neutral (pH = 6,71 – 6,98). In Table 4 are presented obtained results from analyzes of macroelements nitrogen, phosphorous and potassium in a soil samples with coleseed. Comparing total nitrogen content in coleseed and wheat soil samples, it can be estimated that with all fertilization variants nitrogen quantity in case of coleseed soil samples is lower. The reason for this small amount of nitrogen is the great need of coleseed plants of the element (Ivanova, 2012). Macroelement content in case of coleseed samples varies in a very narrow range – from 2,18 g/kg with UAN fertilization (with highest value) up to 2,08 g/kg with

ammonium nitrate fertilization (with lowest value) as differences are proved. Like wheat, values for total phosphorous content in coleseed are highest when DAP is applied (910,34 mg/kg), followed by variants with MAP and NPK (883,54 mg/kg and 871,42 mg/kg respectively). Negligible differences in total phosphorous content are observed with soil samples treated with the rest fertilizer products with minimal value when ammonium nitrate is applied. Statistical analyze estimates proved differences in macroelement content between all fertilization variants. It is obvious that data variations between repetitions are extremely low.

cultures. Levels of this enzyme are higher in fertilization with ammonium nitrate and NPK. Highest catallase values in samples with NPK correlated with higher potassium content in soil samples with both cultures. The highest catallase activity is observed in cases when wheat is fertilized with urea. The lowest catallase activity is observed when DAP is applied at both crops, as well as with UAN usage for wheat fertilization. Lower is the activity as well with non fertilized soil sample. Fertilization with MAP shows average values for catallase activity compared with rest fertilizers with both crops. Catallase activity is higher in wheat and lower in coleseed (excluded sample fertilized with UAN).

Table 5. Catallase activity of soil microorganisms

N _o	Crop	Variant fertilizers	ml 0 ₂ /30 min. Mean ± SD
Control case	Without crop – soil sample at the beginning of the experiment (soil + substrate)	Without fertilizer	1,30 ± 0,05
1	Wheat	NH ₄ NO ₃	1,60 ± 0,05
2		DAP	1,33 ± 0,06
3		MAP	1,52 ± 0,03
4		UAN	1,28 ± 0,03
5		Urea	1,67 ± 0,06
6		NPK	1,60 ± 0,10
1	Coleseed	NH ₄ NO ₃	1,57 ± 0,06
2		DAP	1,28 ± 0,03
3		MAP	1,42 ± 0,06
4		UAN	1,50 ± 0,05
5		Urea	1,40 ± 0,05
6		NPK	1,58 ± 0,03

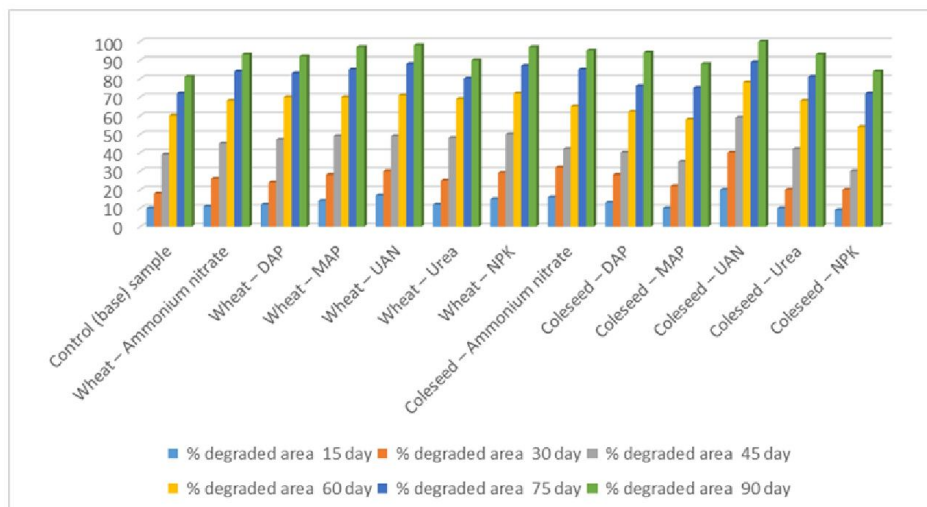


Figure 1. Cellulase activity of soil microorganisms

Total content of potassium resulted from analyzes of soil samples with coleseed shows the biggest value for NPK application case (572,72 mg/kg) where macroelement is a constituent of the product. Statistically is proved that fertilization with urea (552,75 mg/kg) is in second place. At all rest fertilizer products lower than above described values are estimated. Similarly at nitrogen, phosphorous and potassium content in the soil samples with coleseed are lower than their content in soil samples with wheat. This fact can be explained by already described under chapter Introduction ability of this crop to "export" more nutrients from the soil. Soil reaction for all samples is from slightly acidic to neutral (pH=6,41-7,00). Usage of these fertilizers doesn't lead to substantial change in a pH value as this is beneficial for plant growth. Results for catallase activity of soil samples are presented in Table 5. The tendency in terms of catallase activity is the same with both

This tendency correlates generally with higher content of analyzed macroelements – nitrogen, phosphorous and potassium in wheat soil samples compared with coleseed samples. According to research of Rodríguez-Kábana and Truelove (1982) higher average values of catallase are registered in a soil under wheat and soybean, and lower enzyme activity is estimated in a soil under corn and cotton. The mode of fertilization affected soil catallase regardless of harvest. Lacking of some main nutrient in the soil leads to decreasing of enzyme activity. The highest catallase activity is estimated in a soil fertilized with P and K and N. Catallase activity is decreased when additional quantities of mineral nitrogen are added to the established regime. Uzun and Uyanöz (2011) find that soil pH (neutral reaction) influences negligible but positive on the enzymes activity (urease and catallase), while organic matter and water soluble phosphorous content

posses significant positive correlation with these enzymes. Also, the fertilization regime and crops rotation influence of soil catalase. According these authors the activity of catalase soil enzyme depends on clay content, soil moisture, soil depth, soil temperature, organic matter, pH, nutrients, microbe content and activity. Fertilization with selected products increases the activity of cellulase by up to 19% (Figure 1). Cellulase activity is highest at both crops when UAN is applying. It is observed that degradation of colesed's filter leafs is full - 100%. With wheat, the other two fertilizers that provide higher cellulase activity are MAP and NPK. It is noteworthy that fertilization of colesed with same products decreases cellulase activity. The rest of the fertilizer products determine more than 90% degraded area at 90th day with both crops. The lowest cellulase activity is observed in non-fertilized sample (control case).

According to research of Xiao *et al.* (2015) cellulase activity is more dependent on carbon, nitrogen, phosphorous and potassium content in the soil. In contrary, the correlation between catalase activity and content of phosphorous and potassium is significantly lower. Some previous studies have shown that nitric fertilization can increase the activity of cellulase enzymes (Sinsabaugh *et al.*, 2005), urease enzymes (Saiya-Cork *et al.*, 2002) and phosphatase enzymes (Guo *et al.*, 2011; Saiya-Cork *et al.*, 2002), but sometimes can decrease the activity of urease (Burket and Dick, 1998), cellulase (Sinsabaugh *et al.*, 2005), peroxidase (DeForest *et al.*, 2004) and protease (Giacometti *et al.*, 2013). Mineral nitrogen can directly affect microbe synthesis of soil enzymes. The results vary depending on soil and enzyme types, as well as on enzymatic reaction type (Iyyemperumal and Shi, 2008). On the other hand nitric fertilization, especially in mineral forms, can have an indirect effect on the activity of soil enzymes via changes of soil properties, such as changing soil reaction (Gianfreda and Ruggiero, 2006). More frequently the enzymatic activities are increased when fertilization with organic and inorganic nitrogen fertilizers is applied together (Eivazi *et al.*, 2003).

Conclusion

Content of total nitrogen, phosphorous and potassium is higher in fertilized soil samples with wheat comparing with colesed. This fact proves higher ability of colesed to extract nutrients from soil and respectively its higher needs from nutrients. Everything said above must be taken into account when optimal norms for fertilization are determined with both crops. In a test with common winter wheat, highest content of total nitrogen and phosphorous is achieved when DAP is applied. The highest content of total potassium is estimated with fertilization with complex fertilizer NPK 14:14:14 + 11,5S. The achieved results for maximal values of total potassium and phosphorous content in colesed are identical with wheat. When fertilization with liquid nitrogen fertilizer (UAN) is applied, the content of total nitrogen in colesed is highest compared to the rest variants.

Catalase activity of all tested samples is higher in a soil with wheat in comparison with soil samples with colesed. This corresponds to a higher nitrogen, phosphorous and potassium content in wheat. Catalase content is higher in case of fertilization with ammonium nitrate and NPK and is lower when DAP is applied for both crops. The activity of cellulase enzyme is high when fertilization with above described

preparations is applied, as the best result is achieved with UAN application with both crops. High cellulase activity in wheat is achieved when fertilization with MAP and NPK is done. Applying of these products with colesed leads to lower cellulase activity. Fertilization with examined products increases soil enzymatic activity. Higher are values with catalase and cellulase in case of fertilized soil samples compared with control non fertilized sample. These enzymes can serve as specific biochemical markers of fertilized soils.

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