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Influence of Tytanit® and EM on biochemical, physiological, and qualitative parameters of common bean

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Abstract: The role of preparations supporting plant growth is mainly to reduce the harmful effects of various stress factors on plants and to ensure high yields of good quality. This experiment compared the effect of the mineral stimulator Tytanit® and the biological preparation Effective Microorganisms (EM) on the physiological and biochemical activity, as well as the yield of the common bean (*Phaseolus vulgaris* L.). The photosynthetic pigments, free proline and malondialdehyde were assayed and compared at three phenological phases of the bean: 15 BBCH, 24 BBCH, 65 BBCH. The yield parameters included the average number of pods per plant, as well as their fresh and dry mass. Additionally, the nutrient content in the pods was determined according to the atomic absorption spectrometry method. The study revealed a positive effect of both preparations on increasing the content of chlorophyll *a*, *b*, and the carotenoids in the bean leaves. Plants treated with Tytanit® were characterised by the highest content of malondialdehyde and proline, while EM maintained the aldehyde content on a similar level compared to the untreated plants and significantly reduced the proline content. Both preparations significantly decreased the Mn, Mg, P, and Ca content in the pods and did not have a substantial impact on the yield.

Keywords: pigments; proline; malondialdehyde; nutrients; yield

Progressing climate changes and weather anomalies affect the occurrence and intensity of abiotic stress. Decreases in the yield and quality of crops are some of the consequences. Therefore, in modern agriculture, much attention is paid to the use of preparations that support plant productivity and the quality of the obtained yield, i.e., mineral stimulators, biopreparations enriched with useful microorganisms or natural plant extracts (Sas Paszt et al. 2015).

Effective Microorganisms (EM) and Tytanit® (T) belong to two separate groups of preparations supporting plant growth. EM are biological preparations consisting of selected, naturally occurring microorganisms, such as *Lactobacillus case*, *Rhodopseudomonas palustris, Saccharomyces albus, Streptomyces albus* and *Aspergillus oryzae* (Higa 2004). In turn, Tytanit® is a mineral plant growth stimulator, the main component of which is titanium 0.8% Ti.

The microorganisms contained in EM preparations have a high ability to produce antioxidants, thus naturally supporting the defence system of plants. They have a positive effect on the soil structure and on the plants' acquisition of available minerals (Higa 2004). Tytanit® increases the activity of iron ions, the vigour of pollen grains, the rate of nutrient uptake and improves the plant health status (Michalski 2008).

Most of the research conducted, so far, has mainly focused on the assessment of the influence of preparations on the yield and quality of the crops. There are few reports describing the direct effect of these preparations on the physiological and biochemical

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characteristics of plants, which determine their productivity. The common bean (*Phaseolus vulgaris* L.) is one of the most economically important cultivated plants in the world, as well as the most frequently used test plant for scientific research.

The aim of the experiment was to study the physiological and biochemical response of the common bean to two different preparations supporting plant growth during the crop cycle. In addition, their impact on the qualitative features of the harvest was determined to facilitate the process in developing strategies using these preparations in agriculture.

MATERIAL AND METHODS

Plant material and experimental design. The experiment was conducted in 2016–2017 from July until the end of September in the vegetation hall of the West Pomeranian University of Technology in Szczecin (53°N).

The experimental material was the green-pod common bean (*Phaseolus vulgaris* L.) var. Jagusia. The beans were sown in 30 cm pots (volume of 4 L), three seeds per pot. The soil was characterised by the granulometric composition of clay sand and the content of organic carbon – 8.7 g C/kg. The pH of the medium was 7.3 and the salinity was 0.24 g/L NaCl. The mineral composition is shown in Table 1.

According to Szafirowska and Kaniszewski (2014), the optimal content of the basic nutrients for beans should be: N approx. 30, P 40–60, K 125–175, Mg 50–70, Ca 1 000–2 000 mg/L soil. The soil used in the experiment was characterised by a high content of P and K and a low content of N. Beans have the highest demand for K and P, and a lower demand for N. Considering the ability of beans to bind N from the air, no fertilisers were used in the experiment.

A two-factor pot experiment was set up following a randomised complete block design with three replications. The first factor included 3 levels: 1 – application of the EM (Effective Microorganisms, Greenland Technology EM™, Poland); 2 – application of T (Tytanit®, Intermag, Poland), and 3 – control, with no treatment. The second factor was the date of measurement (3 levels): 15 BBCH – 5 leaves developed, 24 BBCH – visible fourth shoot and 65 BBCH – full flowering phase, 50% of open flowers.

Aqueous solutions of EM and T were sprayed on to the bean plants in the following manner: EM 2 L/ha in phases 13 BBCH – 3 leaves developed, 23 BBCH – third shoot developed, 29 BBCH – 9 shoots developed; T-0.2 L/ha in phases 13 BBCH and 23 BBCH.

METHODS

The pigment content: chlorophyll-*a* (chl *a*) and chlorophyll-*b* (chl *b*) were determined according to Arnon's method (1956) as modified by Lichtenthaler and Wellburn (1983). The carotenoids were analysed according to the Hager and Mayer-Berthenrath method (1966).

The concentration of the free proline in the fresh common bean leaves was determined using the Bates et al. (1973) method.

The concentration of malondialdehyde (MDA) was determined by a slightly modified method according to Sudhakar et al. (2001). The determinations for the proline and MDA were conducted via a Shimadzu 1800 UV-Vis spectrophotometer.

The pods were collected by the end of September at the stage of phenological maturity. The average number per plant, their fresh and dry mass were determined. The contents of the selected forms of macroelements Mg, K, Ca, Na, P and microelements Fe and Mn were determined by the atomic absorbance spectrometry method according to Sapek and Sapek (1997).

The results of the study were subjected to a multifactor analysis of variance (ANOVA). Homogeneous groups were determined using Tukey's test at a significance level of P = 0.05. The results presented in all the tables are the averaged results from the gathered data.

RESULTS AND DISCUSSION

Photosynthetic pigments. The plants treated with Tytanit® were characterised by the highest mean content of assimilation pigments. The lowest content of the discussed pigments was observed in the plants from the control group (Table 2). The statistical analysis showed a significant influence of the studied factors on the content of the photosynthetic

Table 1. Mineral composition of the soil used for the experiment (mg/L).

N-NO3	P	K	Ca	Mg	Na	Cl	Mn	Cu	Zn	Fe
17	160	184	1 232	88	18	21	17.6	2.9	7.2	89.7

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Table 2. Effect of the performed treatments on the pigment content, malondialdehyde (MDA), and proline in the fresh leaves of the common bean, measured at different phenological phases

Treatment	Term			Average
	15 BBCH	24 BBCH	65 BBCH	
Chl *a* (mg/g FW)				
C	1.609^{bc}	1.600^{bcd}	1.568^{bcd}	1.592^{B}
EM	1.672^{abc}	1.275^{d}	2.001^{a}	1.649^{AB}
T	1.945^{a}	1.493^{cd}	1.832^{ab}	1.757^{A}
Average	1.742^{A}	1.456^{B}	1.800^{A}	
Chl *b* (mg/g FW)				
C	0.681^{abcd}	0.583^{cd}	0.600^{cd}	0.621^{B}
EM	0.741^{ab}	0.555^{d}	0.776^{a}	0.693^{A}
T	0.782^{a}	0.633^{bcd}	0.713^{abc}	0.707^{A}
Average	0.733^{A}	0.590^{B}	0.698^{A}	
Carotenoids (mg/g FW)				
C	0.811^{bcd}	0.801^{cd}	0.846^{bcd}	0.819^{B}
EM	0.946^{abc}	0.664^{d}	1.125^{a}	0.912^{A}
T	1.077^{a}	0.770^{cd}	1.021^{ab}	0.956^{A}
Average	0.945^{A}	0.745^{B}	0.997^{A}	
MDA (nmol/g FW)				
C	38.22^{b}	21.89^{cd}	20.60^{cd}	26.91^{B}
EM	43.22^{a}	20.97^{cd}	18.10^{e}	27.43^{AB}
T	41.33^{a}	22.62^{c}	19.96^{de}	27.97^{A}
Average	40.93^{A}	21.83^{B}	19.56^{C}	
Proline (μmol/g FW)				
C	0.232^{c}	0.239^{c}	0.663^{b}	0.378^{A}
EM	0.139^{d}	0.122^{d}	0.701^{ab}	0.321^{B}
T	0.230^{c}	0.161^{cd}	0.756^{a}	0.382^{A}
Average	0.201^{B}	0.174^{B}	0.707^{A}	

The means with the same letter were not significantly different by Tukey's comparison at a $P < 0.05$ level; C – control; EM – Effective Microorganisms; T – Tytanit®; FW – fresh weight

pigments in the bean leaves. The greatest influence on the content of chl *a*, chl *b* and the carotenoids was found at the time of measurement, p = 37.20%, p = 42.32%, p = 42.95%, respectively (Table 3). In the control variant, the measurement time did not have a significant effect on the content of the tested pigments. However, the use of Tytanit® (T) and EM preparations caused a significant decrease in the content of the assimilation pigments in the second measurement period and their repeated significant increase in the third period when the highest content was recorded. Particularly high values of these pigments were observed in the plants treated with EM (chl *a* = 2.001 mg/g, chl *b* = 0.776 mg/g, carotenoids = 1.125 mg/g).

The interaction between the applied preparations and the time had a significant effect on the content of chl *a* and the carotenoids (p = 31.50% and p = 22.78%). A smaller, but also significant influence of the application of preparations was observed (p = 7.62% and p = 11.75%).

The application of the preparations had a greater influence on the content of chl *b* than the interaction. The value of the error ranging from 22.52% to 26.11% indicates a significant influence of factors not studied in the experiment, i.e., the temperature, light, humidity or pollutants in the soil.

The positive effect of titanium on the increase in the content of the assimilation pigments was reported by Carvajal et al. (1994); Hrubý et al. (2002);

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Table 3. ANOVA for the measured pigments and oxidative stress indicators

	Chl *a*		Chl *b*		Carotenoids		Proline		MDA	
	P	*p* (%)	*P*	*p* (%)	*P*	*p* (%)	*P*	*p* (%)	*P*	*p* (%)
a	0.023	7.62	0.001	16.35	0.003	11.75	0.000	1.25	0.034	0.20
b	0.000	37.20	0.000	42.32	0.000	42.95	0.000	95.58	0.000	97.24
a × *b*	0.000	31.50	0.012	15.23	0.001	22.78	0.000	1.75	0.000	1.86
Error		23.68		26.11		22.52		1.43		0.70
Total		100		100		100		100		100

a – treatments; *b* – measurement date; *a* × *b* – interaction of tested factors; *P* – probability of error; *p* (%) – percentage of contribution

Wadas and Kalinowski (2017). Titanium improves the absorption of iron, which is an important factor of chlorophyll synthesis (Hrubý et al. 2002). In turn, EM have the ability to increase the efficiency of photosynthesis, which is directly related to the content of the photosynthetic pigments (Ragab et al. 2010; Talaat 2014). The high concentration of carotenoids in the plants treated with Ti could be a response to the suppressed reactive oxygen species by heavy metals (Samadi et al. 2015).

An increase in the content of the tested pigments in the case of the plants treated with T and EM during the period of pod formation is most likely a result of the higher demand of the plants for the assimilates allocated in the forming pods and seeds, and, thus, a related increase in the intensity of the assimilation processes and photosynthetic pigments directly influencing these processes. T and EM seem to be activating the synthesis of the pigments.

Free proline. The highest mean proline content was found in the plants treated with T and the control plants (0.382 and 0.782 μmol/g FW). Significantly lower levels were seen in plants treated with EM 0.321 μmol/g FW (Table 2).

A rapid increase in the proline content was observed in all the studied variants at the end of the vegetation period. These results were confirmed by Auriga and Wróbel (2018) in an experiment with basil, where it was shown that older plants were characterised by a significantly higher proline content.

The study showed a significant influence of the applied preparations and the measurement date on the proline content in the plant tissues (Table 3). The measurement date p = 95.58% had the most significant effect and the lowest use of the preparations p = 1.25%.

The lower average proline content in the plants treated with EM indicates its counteracting effect on the stress reactions, which are the result of various stress factors occurring during ontogenesis or the ageing process itself. However, the highest mean proline content in the variant with T might be a result of an intolerance to Ti by the plant.

Malondialdehyde (MDA). The highest mean MDA content was recorded in the first term of the measurement with 40.93 nmol/g FW In the second term, the mean content of the dialdehyde decreased by almost 50%, while, in the third term, the lowest values for all the variants were recorded (Table 2). Similarly, as in the case of proline, the content of MDA in the fresh plant tissue was influenced by all the studied factors, and the most significant influence was found for the time of measurement p = 97.24%, and the lowest for the use of the preparations p = 0.20% (Table 3).

High levels of MDA in the 15 BBCH phase indicate the occurrence or emergence of a stress factor. However, the observed high average MDA content in the beans treated with these preparations compared to the control may indicate their adverse effect on this species and an oxidative stress induction. Experiments conducted by Talaat (2014) on beans and Auriga and Wróbel (2018) on basil showed a significant effect of the EM on the reduction of the MDA levels in plant tissues, which confirms the mitigating effect of the preparation and is consistent with the results of our experiment.

High levels of MDA in plants treated with T were also noted by Ghosh et al. (2010). They showed an almost 5-fold increase of the MDA concentration in *Allium cepa* roots after application of TiO_2. The authors suggested that TiO_2 nanoparticles may indirectly lead to an excessive production of peroxide radicals, which results in increased lipid peroxidation and oxidative stress.

Nutrient content. The statistical analysis revealed a negative effect of the T and EM on the nutrient composition of the bean pods. Both preparations

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Table 4. Nutrient content in the dry weight of the common bean pods

	Nutrients (g/kg DW)						
	Na	Ca	K	Fe	Mn	Mg	P
C	0.693[a]	3.630[a]	11.921[a]	0.045[a]	0.017[a]	1.602[a]	5.770[a]
EM	0.645[a]	3.396[ab]	12.060[a]	0.029[b]	0.015[b]	1.392[b]	5.126[b]
T	0.241[b]	3.129[b]	10.941[a]	0.044[a]	0.013[b]	1.295[b]	5.598[ab]

The means having the same letter were not significantly different by Tukey's comparison at a $P < 0.05$ level; C – control; EM – effective microorganisms; T – Tytanit®; DW – dry weight

significantly decreased the content of Ca, Mn, Mg and P. Additionally, Tytanit®, significantly lowered the content of Na in the pods, whereas EM significantly lowered the content of Fe in comparison to the control (Table 4).

Complementing the deficits of micro- and macroelements that commonly occur in the human population is one of the major problems of present times. The best assimilable source are plants with high biological values. Moraghan and Grafton (2001) showed the dependence of Fe, K, Mg, Mn, Ca, Na accumulation in asparagus beans on the cultivation site, substrate composition and individual genetic traits of the cultivar. Similar observations were made by Golam Masum Akond et al. (2011), who attributed the ability to accumulate specific micro- and macroelements to specific bean genotypes.

Even though some studies have reported increased mineral contents in plant tissues under the influence of Ti, there are a lack of reports regarding the common bean. Kužel et al. (2003) found an increased content of Fe in oat plant tissues. According to the authors, Ti may cause an apparent deficiency of Fe (and possibly also Mg), contributing to the higher absorption of Fe and other metals by the roots. In the present study, all the nutrient concentrations in the T treated plants were lower compared to the control group. This may indicate the toxic influence of Ti on the Jagusia variety. Low doses of Ti may be beneficial to the plant, but at higher doses, it may be toxic. Therefore, the positivity or negativity of the effect on the particular parameter is dose-responsive and depends on the strength of the plant defence reaction versus the Ti toxic effect on the particular parameter (Kužel et al. 2003).

Talaat et al. (2015), in an experiment with the common bean, showed a positive influence of the application of EM on the elemental composition of the seeds. The content of the individual elements in the seeds was, on average, 5 to 10% higher compared to the variant not treated with the EM. Additionally, the authors noted that the Na content in the seeds decreased by about 8%. However, this was not seen in our study.

Yield. The statistical analysis did not show any significant influence of the treatments on the number of pods, as well as the fresh and dry mass of pods (Table 5). However, the control group was characterised by a higher average number of pods per plant and a higher dry mass (12.75 pods and 11.25 g d.m.).

These results corroborate the findings of Martyniuk and Księżak (2011), who indicated no effect of the EM preparations on the maize yield and selected elements of the yield structure. Similar results were reported by Nowacki et al. (2010) in winter rye, oats, pellets and potatoes.

Szewczuk and Juszczak (2003) showed a 30% increase in the yield of tickled beans under the influence of T, similarly to a study with asparagus beans, where a significant increase in the commercial yield and the number of pods after the application of EM was seen (Pniewska 2014). On the other hand, Kucharski and Jastrzębska (2005) showed that EM-1

Table 5. Effect of the performed treatments on the yield of the common bean

	No. of pods/plant		FW of pods (g)/plant		DW of pods (g)/plant	
	average	std. dev.	average	std. dev.	average	std. dev.
C	12.75[a]	2.19	110.72[a]	14.86	11.25[a]	1.77
EM	11.33[a]	1.87	102.16[a]	10.24	10.34[a]	1.38
T	11.67[a]	2.65	100.50[a]	13.67	10.25[a]	1.64

C – control; EM – effective microorganisms; T – Tytanit®; No. – number; FW – fresh weight; DW – dry weight

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and EM-2 can have a negative impact on the lettuce growth and development.

Yielding is the ability of the plants to produce biomass for specific economic uses. It is determined by the genetic features of the plants, soil and climate conditions, physiological processes in the vegetation cycle and cultivation technology. However, photosynthesis plays one of the most crucial roles during the production of the biomass and agricultural crop. In the present study, plants treated with EM or T were characterised by high concentrations of photosynthetic pigments, which secured the synthesis of the organic compounds and thereby, the overall biomass build-up. Nevertheless, the insignificant decline in the number of pods, their fresh weight and dry weight per treated plant could be the result from the different dosage requirements of T or EM for the Jagusia variety compared to general recommendations for the common bean.

CONCLUSION

The results obtained in this experiment revealed the impact of Tytanit® and Effective Microorganisms on the green-pod common bean var. Jagusia. The preparations positively affected the content of chlorophyll *-a*, *-b*, and the carotenoids. In contrast to Tytanit®, EM indicated oxidative stress-reducing properties. However, none of the biochemical and physiological responses of the plant reflected in an increase in the size and quality of the yield. Moreover, the nutrient content in the treated pods was significantly lower than in the untreated pods. Therefore, the application of the preparations in the cultivation of this particular green-pod common bean variety might not be suitable, and the effectiveness of the preparations may be dependent on the variety.

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