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Rozprawa doktorska

Ocena oddziaływania preparatów EM i Tytanit® na kształtowanie się parametrów biochemicznych, fizjologicznych i jakościowych wybranych roślin ogrodniczych

Evaluation of the impact of EM and Tytanit® preparations on the biochemical, physiological and quality parameters of selected horticultural plants

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Podziękowania

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Streszczenie

Stosowanie biostymulatorów w uprawach ogrodniczych wpływa na ograniczenie chemizacji produkcji roślin przy jednoczesnym utrzymaniu wydajności i jakości plonów oraz zachowaniu zasobów środowiska naturalnego. Należy jednak zaznaczyć, że biostymulatory nie są uniwersalne, a ich dobór do uprawianego gatunku jest uzależniony od takich zmiennych jak m. in. czynniki abiotyczne czy uwarunkowania genetyczne roślin. Dotychczasowe badania nad zastosowaniem biostymulatorów skupiały się głównie na ich wpływie na produktywność i wielkość plonowania, natomiast w mniejszym stopniu analizowano ich oddziaływanie na procesy biochemiczne i fizjologiczne roślin ogrodniczych. Efektywne Mikroorganizmy (EM) i Tytanit® należą do grupy biostymulatorów cieszących się coraz większym zainteresowaniem producentów żywności, jednak wiedza o ich wpływie na konkretne gatunki i odmiany roślin użytkowanych gospodarczo jest podstawowa i wymaga uzupełnienia.

W ramach niniejszej pracy przeprowadzono cztery niezależne eksperymenty na wybranych gatunkach roślin ogrodniczych. Zastosowanie EM w uprawie bazylii pospolitej (*Ocimum basilicum* L.) odmiana Piccolino spowodowało obniżenie dwóch badanych wskaźników stresu oksydacyjnego tj. proliny i dialdehydu malonowego. W przypadku uprawy dwóch odmian winorośli (*Vitis vinifera* L.) Regent i Cabernet Cortis prowadzonych na 4 i 8 pędów, EM nie miały istotnego wpływu na zawartość ekstraktu ogólnego oraz kwasowość owoców, natomiast wpłynęły na obniżenie w owocach ogólnej zawartość polifenoli. Eksperyment dotyczący zastosowania Tytanitu w uprawie poziomki pospolitej (*Fragaria vesca* L.) odm. Baron von Solemacher uprawianej w warunkach zasolenia wykazał zróżnicowany wpływ preparatu na zbadane parametry fizjologiczne. Na początku okresu wegetacji Tytanit® wpłyną na obniżenie poziomu proliny w roślinach, natomiast w późniejszym okresie obniżył wartości wskaźników wydajność aparatu fotosyntetycznego roślin Fv/F_M i Fv/Fo, a także spowodował zmniejszenie zawartości barwników asymilacyjnych. Na podstawie uzyskanych wyników można wnioskować, że preparat nie wykazał łagodzącego wpływu na stres wywołany zasoleniem, jak zakładano. Eksperyment z fasolą zwyczajną (*Phasoleus vulgaris* L.) odm. Jagusia, wykazał pozytywny wpływ obu preparatów tj. EM i Tytanitu na zwiększenie zawartości barwników asymilacyjnych. Efektywne Mikroorganizmy w przeciwieństwie do Tytanitu, zredukował syntezę proliny i dialdehydu malonowego. Zastosowane preparaty nie miały wpływu na wielkość plonu, natomiast istotnie obniżyły jego jakość poprzez zmniejszenie zawartości manganu, magnezu, fosforu oraz wapnia w strąkach.

Abstract

The use of biostimulants in horticultural reduces the chemization of plant production while maintaining yield and quality of crops and preserving natural resources. However, it should be noted that plant biostimulants are not universal and their use for a species depends on many variables such as abiotic factors and genetic variations. Previous studies on the use of biostimulants have focused mainly on their impact on productivity and yielding, while their impact on the biochemical and physiological traits of horticultural plants has been analyzed to a lesser extent. Effective Microorganisms (EM) and Tytanit® are biostimulants that become more popular among food producers, but the knowledge about their influence on specific species and varieties of plants is basic and requires supplementation.

Four individual experiments were carried out on selected species of horticultural plants. The use of EM in the cultivation of sweet basil (*Ocimum basilicum* L.), var. Piccolino, resulted in the reduction of two analyzed parameters related to oxidative stress, i.e. proline and malondialdehyde contents. In the case of the cultivation of two grapevines (*Vitis vinifera* L.) cv. Regent and Cabernet Cortis rooted with 4 and 8 buds, EM had no significant effect on the total extract content and fruit acidity. However, it reduced the overall content of polyphenols in the fruit. The experiment on the use of Tytanit® in the cultivation of wild strawberry (*Fragaria vesca* L.) var. Baron von Solemacher under salinity conditions, showed varied effect on the studied physiological parameters. At the beginning of the growing season, Tytanit® lowered the level of proline in plants, while later it lowered the efficiency of the photosynthetic apparatus of plants Fv/F_M and Fv/Fo, and reduced the content of assimilation pigments. On the basis of the obtained results, it can be concluded that the preparation did not show an alleviating effect on salinity stress, as assumed. The experiment with common beans (*Phasoleus vulgaris* L.) var. Jagusia, revealed a positive effect of both preparations on photosynthetic pigments. EM, unlike Tytanit®, reduced the synthesis of proline and malondialdehyde. However, the preparations did not affect yield, but significantly decreased its quality by reducing the content of manganese, magnesium, phosphorus and calcium in the pods.

Spis treści

DOROBEK NAUKOWY STANOWIĄCY ROZPRAWĘ DOKTORSKĄ

Ocena oddziaływania preparatów EM i Tytanit® na kształtowanie się parametrów biochemicznych, fizjologicznych i jakościowych wybranych roślin ogrodniczych

Lp.	Tytuł publikacji	Pkt.*	IF**
P1	**Auriga A.,** Wróbel J. (2018) Effect of effective micro-organisms on the proline and MDA contents in herb plant material of *Ocimum basilicum* L. var. Piccolino. *Fresenius Environmental Bulletin*, vol. 27 (11/2018), 7409-7415	15	0,691
P2	**Auriga A.**, Ochmian I., Wróbel J., Oszmiański J. (2018) The influence of Effective Microorganisms and number of buds per cane in viticulture on chemical composition in fruits. *Journal of Applied Botany and Food Quality* 91, 271 - 280, DOI:10.5073/JABFQ.2018.091.035	25	1,106
P3	**Auriga A.**, Wróbel J., Ochmian I. (2020) Effect of Tytanit® on the physiological activity of wild strawberry (*Fragaria vesca* L.) grown in salinity conditions. *Acta Universitatis Cibiniensis Series E: FOOD TECHNOLOGY,* vol. XXIV, no. 2, 279-288, DOI:10.2478/aucft-2020-0025	140	2,000
P4	**Auriga A.**, Wróbel J. (2021) Influence of Tytanit® and EM on biochemical, physiological, and qualitative parameters of common bean. *Horticultural Science (Prague)* 48, (2): 98 – 104, https://doi.org/10.17221/72/2020-HORTSCI	70	0,925
	Suma	**250**	**4,722**

*Liczba punktów według listy MNiSW zgodna z rokiem ukazania się pracy

**Sumaryczny Impact Factor (IF) według bazy Journal Citation Reports (JCR) z roku wydania

1. Przegląd literatury

Postępująca degradacja środowiska naturalnego jest wynikiem nierozważnej działalności człowieka. Rozwój technologiczny z jednej strony przyczynia się do polepszenia warunków życia, jednak z drugiej strony często niesie ze sobą coraz większe zagrożenie dla otoczenia przyrodniczego (Gołębiewska i Pajewski, 2017).

Sektor rolniczy ze względu na charakter wytwarzanych produktów jest niezwykle istotny dla egzystencji ludzkości. Intensyfikacja upraw i produkcji roślin metodami konwencjonalnymi wiąże się z dużą chemizacją rolnictwa, co bezpośrednio wpływa na jakość żywności oraz ograniczenie zasobów słodkiej wody i przydatnych do upraw areałów. Dodatkowo zachodzące w przyrodzie zmiany klimatu, co za tym idzie, pojawiające się coraz częściej anomalie pogodowe, wpływają na potęgowanie się stresu abiotycznego u roślin, wywołane między innymi suszą, zasoleniem czy niedoborem składników pokarmowych.

Rośliny poddane działaniu długotrwałego stresu są w słabszej kondycji fizjologicznej, dlatego ich plony charakteryzują się mniejszą wydajnością i jakością (Starck i in., 1993). Z tego powodu w nowoczesnym rolnictwie dużo uwagi poświęca się wykorzystywaniu różnorodnych preparatów, które wspomagają produktywność roślin, ale też mają działanie ochronne i jednocześnie nie generują efektów ubocznych, takich jak wyjałowienie czy zakwaszenie gleb. Są to najczęściej biostymulatory lub preparaty wzbogacone pożytecznymi mikroorganizmami (Sas Paszt i in., 2015), naturalnymi wyciągami roślinnymi albo zwierzęcymi (Sas Paszt i in., 2010; Lisiecka i in., 2011). Do oceny kondycji fizjologicznej roślin wykorzystuje się, zarówno wskaźniki biochemiczne, jak i fizjologiczne. Szczególnie przydatne w badaniach ekosystemów i upraw zagrożonych czynnikami fitotoksycznymi i oceny tolerancji roślin na różnorodne czynniki stresowe są metody fluorescencyjne. Mogą one często zastępować bardziej czasochłonne metody gazometryczne stosowane dotąd w fizjologii roślin (Kalaji i Łoboda 2010).

Według rozporządzenia Parlamentu Europejskiego i Rady Unii Europejskiej z 2019 r. biostymulatorem określa się produkt, który stymuluje procesy odżywiania roślin niezależnie od zawartości składników pokarmowych w produkcie. Jego rolą jest poprawa co najmniej jednej z następujących właściwości roślin lub ryzosfery roślin: efektywności wykorzystania składników pokarmowych; cechy jakościowej; przyswajalności składników pokarmowych z form trudnodostępnych w glebie lub ryzosferze. Rozporządzenie wskazuje również podział biostymulatorów na mikrobiologiczne i niemikrobiologiczne. Pierwsze

składają się z mikroorganizmu lub konsorcjum mikroorganizmów, a poziom poszczególnych patogenów w preparatach jest ściśle określony przez ww. rozporządzenie. Natomiast wszystkie biostymulatory, które nie są zaliczane do grupy mikrobiologicznych, stanowią grupę biostymulatorów niemikrobiologicznych.

Efektywne Mikroorganizmy™ to nazwa handlowa biostymulatorów mikrobiologicznych, w których skład wchodzą specjalnie wyselekcjonowane, naturalnie występujące drobnoustroje, takie jak np.: bakterie mlekowe (*Lactobacillus casei, Streptococcus lactis*), bakterie fotosyntetyzujące (*Rhodopseudomonas palustrus, Rhodobacter spae*), drożdże (*Saccharomyces albus, Candida utilis*), promieniowce (*Streptomyces albus, S. griseus*) oraz grzyby pleśniowe (*Aspergillus oryzae, Mucor hiemalis*) (Higa i Parr, 1994; Janas, 2009; Hu i Qi, 2013). Technologia EM została opracowana w latach 80. XX wieku przez japońskiego profesora ogrodnictwa Teruo Higa, który uważał, że w przyrodzie decydującą rolę odgrywają mikroorganizmy (Higa 2004).

Mikroorganizmy znajdujące się w preparatach EM posiadają duże predyspozycje adaptacyjne. Tworzą one dwuwarstwowe kapsuły żelowe ze środowiskiem tlenowym na zewnątrz a beztlenowym wewnątrz. Strefą oddzielającą te dwa środowiska są organizmy fakultatywne, które po stronie zewnętrznej prowadzą metabolizm aerobowo, a po stronie wewnętrznej anaerobowo (Schneider 2005). Obecne w preparacie bakterie fotosyntetyczne mają zdolność wytworzenia przeciwutleniaczy, cukrów, aminokwasów, a także innych substancji bioaktywnych, które korzystnie wpływają na wzrost roślin. Antyoksydanty wytwarzane przez EM naturalnie wspierają system obronny roślin przeciwdziałając wolnym rodnikom, które są główną przyczyną wielu chorób (Higa, 2004; Mrugalska i in., 2009; Małuszyńska i in., 2012).

Działanie preparatów EM nie jest jednoznaczne. W literaturze przedmiotu można znaleźć doniesienia, zarówno o pozytywnych, jak i negatywnych skutkach stosowania tych biostymulatorów. Hui-lian (2000) oraz Chaudhry (2005) wykazali korzystny wpływ EM na uprawę kukurydzy. Podali oni, że preparat stymulował wzrost roślin, indukował ich odporność i procesy fotosyntezy. Z kolei brak istotnego wpływu EM na plon kukurydzy stwierdzili Priyadi i in. (2005). Badania prowadzone przez Prisa (2019a) w uprawie papryki i chili wykazały znaczące zwiększenie biomasy oraz obniżenie zawartości azotanów w roślinach traktowanych EM. Inne badania prowadzone przez Fawzy i in., (2012) oraz Prisa (2019b) wykazały istotny, pozytywny wpływ EM na wzrost roślin cebuli, a także na wielkość oraz jakość uzyskanego plonu. Natomiast EM nie miało wpływu na

plon czy pojawianie się wad bulw ziemniaka (Zarzyńska i Goliszewski, 2011), a także na plon lnu oleistego (Wielgusz i in., 2009).

Jak podają Iriti i in. (2019), zatosowanie preparatów EM w uprawie fasoli zwyczajnej spowodowało zwiększenie liczby ziaren w strąku, liczby ziaren przypadającą na roślinę oraz na zmniejszoną zawartość lipidów i wody w ziarnach. Efektywne Mikroorganizmy wpłynęły również na obniżenie zawartości wapnia, natomiast zwiększyły zwartość magnezu, manganu, fosforu i sodu w ziarnach fasoli. Ponadto, Iriti i in. (2019) u roślin fasoli poddanych działaniu preparatów EM obserwowali wydłużenie o dwa tygodnie optymalnej wydajności fotosyntezy liści w porównaniu do roślin kontrolnych. Badania prowadzone przez Talaat (2015) w warunkach zasolenia wykazały łagodzący wpływ EM na występujący u roślin fasoli stres oksydacyjny, co szczególnie widoczne jest w zwiększonej syntezie białek i zmianie składu poliamin w tkance roślin. Natomiast badania przeprowadzone przez Borowski i Balmonowski (2009) nad zastosowaniem preparatów EM w uprawie bazylii *Ocimum basilicum* L., wykazały znaczny wzrost stężenia proliny w badanym materiale roślinnym, co wskazuje na istotne zaburzenie stanu fizjologicznego rośliny.

Janasa i Grzesika (2005, 2006) wykazali, że biokondycjonowanie preparatem EM nasion wybranych gatunków roślin leczniczych i warzywnych zwiększa zdrowotność nasion oraz poprawia ich wartość siewną. Zastosowanie EM sprzyja także wczesnemu owocowaniu oraz wzrostowi korzeni pomidora (Ncube i in., 2011), natomiast nie sprzyja ogólnemu rozwojowi kapusty pekińskiej (In-Ho i Ji-Hwan, 2012).

Mayer i in (2010) stwierdzili brak wpływu zastosowanych preparatów EM na biomasę drobnoustrojów w glebie, strukturę zbiorowisk drobnoustrojów, parametry aktywności drobnoustrojów w glebie, czy też wielkość plonu w uprawie ziemniaka, jęczmienia ozimego, lucerny oraz pszenicy ozimej.

Według niektórych doniesień EM mają również zdolność hamowania rozwoju patogenów. Biostymulator ograniczył występowanie grzybów z rodzaju *Fosariu* w uprawie lnu włóknistego (Langner i in., 2003) i grzybów z rodzaju *Fusarium* w uprawie grochu siewnego (Okorski i Majchrzak, 2008). W uprawach pszenicy EM chroniły przed rozwojem septoriozy i brunatnej plamistości liści, natomiast nie miały istotnego wpływu na łamliwość źdźbła powodowaną przez grzyby *Pseudocercosporella herpotrichoides* czy na porażenie źdźbła przez zgorzel (*Gaeumannomyces graminis*) (Boligłowa i Gleń, 2008).

Do grupy biostymulatorów niemikrobiologicznych można zaliczyć Tytanit®, którego głównym składnikiem jest tytan (0,85% Ti). Tytan naturalnie występuje

w materiale geologicznym, jest dziewiątym najbardziej rozpowszechnionym pierwiastkiem w skorupie ziemskiej i stanowi ok. 0,57% jej masy (Buettner i Valentine, 2012). Pierwiastek ten jest metalem przejściowym i wykazuje cztery stopnie utlenienia, z których Ti^{4+} jest najbardziej stabilnym jonem. Wpływ tytanu na rośliny jest badany od ponad 100 lat. Pierwsze badania tego pierwiastka w glebie, trzcinie cukrowej (*Saccharum spp.*) i burakach cukrowych (*Beta vulgaris* L.) przeprowadzili Pellet i Fribourg (1905). Jednak do tej pory nie poznano dokładnego mechanizmu oddziaływania Ti na organizmy roślinne.

Prawdopodobny mechanizm działania Ti w organizmie rośliny przedstawili Simona i in. (1988), Carvajal i Alcaraz (1998) oraz Cigler i in. (2010). Wg tych autorów tytan i żelazo (Fe) wpływają na siebie, zarówno synergistyczne, jak i antagonistyczne. Gdy roślina ma niedobór Fe, Ti może indukować ekspresję genów związanych z pozyskiwaniem Fe, zwiększając jego pobieranie i wykorzystywanie, a następnie poprawiając wzrost roślin. Możliwe, że rośliny posiadają białka, które specyficznie lub niespecyficznie wiążą Ti. Gdy stężenie Ti w tkance jest wysokie, pierwiastek może konkurować z Fe o ligandy lub białka. Natomiast wzmożona konkurencja między pierwiastkami może powodować fitotoksyczność Ti. Dlatego korzystne efekty Ti mogą być szczególnie widoczne lub mierzalne w okresie, gdy rośliny doświadczają niedoboru Fe.

Tytan wpływa na metabolizm roślin poprzez zwiększenie wchłaniania innych składników pokarmowych, prócz Fe także Mg (Simon i in., 1988; Kuzel i in., 2003). Pozytywny wpływ tytanu na fotosyntezę oraz aktywność enzymatyczną badanych roślin potwierdzili Carvajal i Alcaraz (1998).

Wpływ tytanu na zwiększenie zawartości chlorofilu *a*, *b* i karotenoidów został stwierdzony przez licznych badaczy (Carvajal i in., 1994; Simon i in., 1988; Hrubý i in., 2002). Samadi i in. (2014, 2015) odnotowali istotny wzrost zawartości karotenoidów w liściach mięty i melisy. Moaveni i in. (2011) zaobserwowali poprawę względnej zawartości wody (RWC) w materiale roślinnym pszenicy.

Grajkowski i Ochmian, (2007) stwierdzili, że oprysk Tytanitem przed zbiorem owoców maliny (*Rubus idaeus* L.) zwiększył zawartość ekstraktu ogólnego w owocach, ich jędrność i wielkość. Z kolei Szewczuk i Juszczak (2003) wykazali 30% zwiększenie plonowania fasoli tycznej uprawianej z wykorzystaniem tego preparatu. Ponadto zastosowanie Ti w uprawie wybranych odmian truskawek miało wpływ na zwiększenie zawartości antocyjanów oraz witaminy C (Skupień i Oszmiański, 2007).

Inne doniesienia naukowe potwierdzają działanie Tytanitu na wzrost aktywności jonów żelaza, zwiększenie wigoru ziaren pyłku i wzrost tempa pobierania składników pokarmowych, a także poprawę zdrowotności roślin (Michalski, 2008; Borkowski i in., 2017).

Zastosowanie preparatów zawierających tytan w uprawie niektórych roślin może mieć również niepożądane skutki. Ghosh i in. (2010) zaobserwowali wysoki (prawie 5-krotny) wzrost stężenia dialdehydu malonowego (MDA) w korzeniach cebuli (*Allium cepa*). Autorzy zasugerowali, że nanocząsteczki TiO_2 mogą prowadzić pośrednio do nadmiernego wytwarzania rodników ponadtlenkowych, co powoduje u roślin zwiększenie peroksydacji lipidów i stres oksydacyjny. Ponadto tytan może wchodzić w interakcję z żelazem w łańcuchu transportu elektronów, co przy wysokim stężeniu tytanu zmniejsza wydajność fotosyntezy II (Cigler i in., 2010).

Właściwy dobór preparatów ze względu na rodzaj uprawy ma kluczowe znaczenie w uzyskaniu, nie tylko wysokiej jakości plonu, ale także dla ochrony środowiska naturalnego. Upowszechnianie niechemicznych metod ochrony roślin przyczynia się do zmniejszenia zależności produkcji roślinnej od stosowania preparatów chemicznych. W rezultacie ryzyko związane z ich użyciem jest ograniczane, co również przekłada się na bezpieczeństwo konsumentów, osób wykonujących zabiegi, a w szczególności wpływa na czystość środowiska (MRiRW, 2019). Według danych Głównego Urzędu Statystycznego od 2010 roku do 2018 roku zużycie środków ochrony roślin ogółem i nawozów mineralnych NPK, zarówno w Polsce jak i całej Unii Europejskiej nieustannie wzrastało. W Polsce w roku gospodarczym 2018/2019 oba wskaźniki zmniejszyły się jednak o ok. 8,5% (środki ochrony) i o ok. 8,24% (nawozy NPK), co może wskazywać na rosnącą świadomość dotyczącą zagrożeń jakie niesie ze sobą nadmierna chemizacja produkcji żywności.

2. Cel i zakres pracy

Celem naukowym pracy było określenie wpływu preparatów EM i Tytanit® na kształtowanie się niektórych parametrów biochemicznych, fizjologicznych i jakościowych wybranych roślin ogrodniczych uprawianych w różnych warunkach siedliskowych.

Celem utylitarnym pracy było określenie przydatności badanych preparatów w uprawie wybranych gatunków roślin ogrodniczych, w aspekcie poprawy jakości plonów w zmiennym klimacie regionu północno zachodniej Polski.

Zakres pracy został podzielony na cztery zadania, które obejmowały:

Zadanie 1. (P1) – analizę wypływu działania preparatu EM na biochemiczne parametry stresu oksydacyjnego, takie jak stężenie wolnej proliny i MDA w świeżych tkankach zielonych bazylii drobnolistnej (*Ocimum basilicum* L.) odmiana Piccolino.

Zadanie 2. (P2) – badanie wpływu EM na zawartość ekstraktu cukrowego, kwasowość i profil polifenoli w owocach dwóch odmian winorośli (*Vitis vinifera* L.) Regent i Cabernet Cortis prowadzonych na różną liczbę pędów, uprawianych w północno-zachodniej Polsce, a także analiza interakcji między badanymi czynnikami i ich wpływu na zawartość poszczególnych grup polifenoli.

Zadanie 3. (P3) – analizę wpływu preparatu Tytanit® na parametry fizjologiczne i biochemiczne: zawartość barwników asymilacyjnych, wydajność aparatu fotosyntetycznego poprzez zmierzenie maksymalnej potencjalnej aktywności fotochemicznej Fv/Fm i maksymalnej efektywności rozszczepienia wody po donorowej stronie PSII Fv/Fo, stężenie wolnej proliny oraz relatywną względną zawartość wody RWC w tkankach zielonych poziomki pospolitej (*Frgaria vesca* L.) odmiana Baron von Solemacher uprawianej w warunkach zasolenia.

Zadanie 4. (P4) – badanie wpływu odziaływania preparatów EM i Tytanit® na parametry fizjologiczne, biochemiczne i jakościowe fasoli zwyczajnej (*Phasoleus vulgaris* L.) zielonostrąkowej, odmiany Jagusia. Badania obejmowały oznaczenie chlorofilu *a* i *b*, karotenoidów, proliny i MDA w liściach roślin oraz badania związane z plonem, tj. oznaczeniem średniej liczby strąków, ich świeżej i suchej masy, a także oznaczenia wybranych mikro i makroskładników.

Określony w pracy cel posłużył do zweryfikowania prawdziwości następującej hipotezy badawczej: zastosowanie preparatów EM i Tytanit® w uprawie wybranych roślin ogrodniczych wpływa na poprawę ich procesów fizjologicznych i plonowania oraz na złagodzenie przebiegu reakcji stresowych wywołanych zasoleniem podłoża, co znajduje odzwierciedlenie w otrzymanych wartościach ich parametrów biochemicznych, fizjologicznych i jakościowych.

3. Materiał i metody badań

3.1. Materiał badawczy

Zadanie 1. (P1). Przeprowadzono dwuletnie doświadczenie wazonowe z bazylią pospolitą (*Ocimum basilicum* L.) odmiany Piccolino z zastosowaniem Efektywnych Mikroorganizmów (EM). Doświadczenie prowadzono w kontrolowanych warunkach wodnych. Materiał roślinny pozyskany w doświadczeniu poddano analizie w laboratorium Katedry Fizjologii Roślin i Biochemii Wydziału Kształtowania Środowiska i Rolnictwa Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie.

Dwuczynnikowe doświadczenie wazonowe założono w układzie bloków losowych w trzech powtórzeniach. Pierwszym czynnikiem były 2 poziomy stosowania EM (1. poziom – wykorzystanie wodnego roztworu EM w rozcieńczeniu 1:100, 2. poziom kontrolny – bez zabiegów z EM). Drugim czynnikiem były 3 poziomy terminów pomiarów.

Nasiona w ilości 10 szt. na wazon wysiano do gotowego podłoża na bazie torfu o pH 5,5 – 6,5, zasoleniu 1,9 g NaCl dm^{-3} i z dawką startową nawozu wieloskładnikowego NPK 14-16-18 w ilości 0,6 kg na m^{-3} podłoża. Materiał roślinny pobrano do badań w odstępach miesięcznych tj.: na początku czerwca, lipca i sierpnia.

Zadanie 2. (P2). Materiał badawczy obejmował dwie odmiany winorośli Regent i Cabernet Cortis. Owoce (grona) do badań pochodziły z 5 letnich roślin uprawianych w nienawadnianej winnicy położonej w Stacji Doświadczalnej ZUT w Ostoi. W czasie wegetacji w 5 i 6 roku uprawy rośliny były traktowane preparatem EM w ilości 10 litrów na hektar, aplikowanym w formie oprysku. Opryski przeprowadzano w dwutygodniowych odstępach w okresie od pojawiania się pierwszych pąków liściowych do dojrzałości fizjologicznej owoców. Dodatkowo rośliny były prowadzone na zróżnicowaną liczbę pędów na łozie tj. 4 i 8. Materiał badawczy stanowiły owoce winorośli zebrane w okresie dojrzałości zbiorczej.

Zadanie 3. (P3). Doświadczenie wazonowe przeprowadzono w hali wegetacyjnej ZUT w Szczecinie, w którym materiałem doświadczalnym była poziomka pospolita (*Fragaria vesca* L.) odmiana Baron von Solemacher, uprawiana w podłożu o trzech poziomach zasolenia z zastosowaniem Tytanitu lub jego brakiem.

W każdym roku nasiona poziomki wysiewano do gotowego podłoża ogrodniczego na bazie torfu i piasku. Podłoże miało pH 6, zasolenie 1,9 g NaCl dm^{-3} i dawkę startową

nawozu wieloskładnikowego o składzie NPK 14-16-18 w ilości 0,6 kg m^{-3}. Po 6 tygodniach młode siewki przepikowano do wazonów o pojemności 1 dm^3 i umieszczono je w osiatkowanej części hali wegetacyjnej. Wilgotność podłoża utrzymywano w zakresie pF 2.2 – 1.7.

Doświadczenie wazonowe założono w układzie bloków losowych w trzech powtórzeniach każde po 8 roślin. Pierwszym czynnikiem były 3 stopnie zasolenia podłoża: S1 - zasolenie gotowego podłoża 32,5 Mm L^{-1} NaCl; S2 - 50 Mm L^{-1} NaCl; S3 - 100 Mm L^{-1} NaCl. Drugim czynnikiem było zastosowanie Tytanitu (T) w jednej dawce w trzech stopniach zasolenia podłoża S1+T, S2+T, S3+T. Rośliny podlewano wodnym roztworem Tytanitu o stężeniu 0,3% w trzech fazach rozwoju roślin: po przyjęciu się sadzonek (rozwinięty 2.–3) liść, rozwinięty 5.–8. liść i na początku kwitnienia, zgodnie z zaleceniami producenta. Kontrolę stanowiły rośliny uprawiane na podłożu o najniższym zasoleniu S1 bez zastosowania Tytanitu. Pomiary badanych parametrów zostały wykonane w dwóch fazach fenologicznych BBCH 15 (rozwinięty 5 liść) i BBCH 60 (otwarte pierwsze kwiaty). Materiał do analiz chemicznych stanowiły liście pobrane ze środka rozety roślin.

Zadanie 4. (P4). Doświadczenie przeprowadzono w hali wegetacyjnej ZUT w Szczecinie, gdzie materiałem doświadczalnym była fasola zwyczajna (*Phasoleus vulgaris* L.) zielonostrąkowa odmiana Jagusia. Nasiona fasoli wysiano do wazonów (3 nasiona/ wazon) o pojemności 4 dm^3. Podłoże stanowiła gleba pobrana z poziomu orno próchnicznego (0÷30 cm) ziem rdzawych. Doświadczenie wazonowe zostało przeprowadzone w układzie dwuczynnikowym z trzema powtórzeniami, w kontrolowanych warunkach wodnych. Badanym czynnikiem było zastosowanie preparatów EM i Tytanit® w sposób i dawkach zalecanych przez producentów zgodnie z wymaganiami uprawianego gatunku; faza 3–5 liścia właściwego (BBCH 13–15) i rozwój pędów (BBCH 21–29).

Pomiary wybranych parametrów wykonano w trzech fazach fenologicznych: 15 BBCH – rozwinięte 5 liści, 24 BBCH – widoczny czwarty pęd i 65 BBCH – pełnia fazy kwitnienia 50% kwiatów otwartych.

3.2. Metodyka badań

W ramach pracy przeprowadzono oznaczenie parametrów biochemicznych, fizjologicznych oraz jakościowych wybranych roślin ogrodniczych.

Badania parametrów biochemicznych obejmowały:

- oznaczenie wolnej proliny wg metody Batesa i in. (1973)
- oznaczenie zawartości dialdehydu malonowego (MDA) wg metody Sudhakar i in. (2001)
- oznaczenie zawartości polifenoli w tym związków należących do antocyjanów, kwasów fenolowych, flawonoli i flawan-3-oli przy wykorzystaniu ultrawydajnej chromatografii cieczowej skojarzonej z detektorem fotodiodowym oraz spektrometrem mas (UPLC-PDA/MS).

Badania parametrów fizjologicznych obejmowały:

- oznaczenie zawartości chlorofilu *a* i *b* oraz karotenoidów wg metody Arnona (1956) zmodyfikowanej wg Lichtenthalera i Wellburna (1983)
- zmierzenie relatywnej zawartości wody w świeżej tkance roślinnej (RWC) wg metody Yamasaki i Dillenbu (1999)
- zmierzenie wydajności aparatu fotosyntetycznego roślin w tym maksymalnej potencjalnej aktywności fotochemicznej (Fv/Fm) oraz maksymalnej efektywności rozszczepienia wody po stronie donorowej PSII (Fv/Fo), metodą fluorescencji chlorofilu *a,* za pomocą fluorymetru Handy-PEA (Hansatech Istruments Ltd. Anglia).

Badania parametrów jakościowych obejmowały:

- oznaczenie zawartości ekstraktu cukrowego za pomocą refraktometru elektronicznego PAL-1 (Atago, Tokyo, Japan)
- oznaczenie kwasowości miareczkowalnej przy użyciu pH-metru (Elmetron 501, Zabrze, Poland), przez miareczkowanie ekstraktu wodnego z 0,1 N NaOH do punktu końcowego pH 8,1
- oznaczenie zawartości składników mineralnych (Na, Ca, K, Fe, Mn, Mg, P) techniką atomowej spektroskopii absorpcyjnej wg. metody Sapek i Sapek (1997)
- określenie średniej liczby strąków z jednej rośliny, a także oznaczenie świeżej i suchej masy strąków fasoli (metodą wagową).

3.3. Analiza statystyczna danych eksperymentalnych

Dane eksperymentalne zostały poddane jedno lub wieloczynnikowej analizie wariancji ANOVA. Obliczono istotność statystyczną poszczególnych czynników jako ich istotność fizyczną przy zastosowaniu procentowego współczynnika wpływu udziału. Istotność różnic między poszczególnymi średnimi i dla interakcji określono za pomocą testu Tukey'a przy poziomie istotności p=0,05. Obliczenia przeprowadzono przy użyciu programów Microsoft Excel i/lub Satistica 12.0.

4. Omówienie uzyskanych wyników

Niniejsza rozprawa doktorska została oparta na czterech oryginalnych artykułach naukowych spójnych tematycznie i opublikowanych w czasopismach znajdujących się na liście MNiSW (P1-P4). Artykuły stanowiące dysertację poruszają problematykę wpływu preparatów EM i Tytanit® na ogólny stan fizjologiczny wybranych roślin ogrodniczych. Pierwsze dwa artykuły dotyczą wpływu preparatu EM na parametry biochemiczne, odpowiednio: ziela bazylii (P1) i owoców winorośli (P2). Dodatkowo w przypadku owoców winorośli oznaczono ich cechy jakościowe, zarówno pod wpływem EM oraz sposobu cięcia i prowadzenia pędów. Wpływ preparatu Tytanit® na parametry biochemiczne i fizjologiczne poziomki pospolitej został przedstawiony w artykule trzecim (P3). Ostatni ze wskazanych artykułów (P4) przedstawia porównanie wpływu preparatów EM i Tytanit® na wybrane parametry biochemiczne, fizjologiczne i jakościowe fasoli zwyczajnej.

Sprawnie przebiegające procesy biochemiczno-fizjologiczne decydują o prawidłowym wzroście roślin, co w konsekwencji wpływa na jakość i wielkość plonu. Dlatego w dysertacji doktorskiej, będącej kompilacją czterech artykułów naukowych skupiono się na analizie wskaźników biochemicznych, fizjologicznych i jakościowych, które wydają się najbardziej adekwatne w ocenie kondycji wybranych roślin ogrodniczych, rosnących w zróżnicowanych warunkach podłoża i poddanych oddziaływaniu biostymulatorów.

4.1. Effect of effective micro-organisms on the proline and MDA contents in herb plant material of *Ocimum basilicum* L. var. Piccolino (P1)

Występowanie stresu oksydacyjnego u roślin jest skutkiem oddziaływania jednego lub wielu niekorzystnych czynników zewnętrznych. Postępująca degradacja środowiska naturalnego, m.in. w wyniku wysokiej chemizacji upraw, a także niekorzystne zmiany klimatu multiplikują ilość tych czynników. Ich występowanie i negatywny wpływ na rośliny wiąże się z koniecznością stosowania przyjaznych dla środowiska preparatów, takich jak np. EM, które wspomagają rozwój roślin i wpływają na ograniczenie występowania stresu oksydacyjnego w czasie ich wzrostu (Higa, 2004).

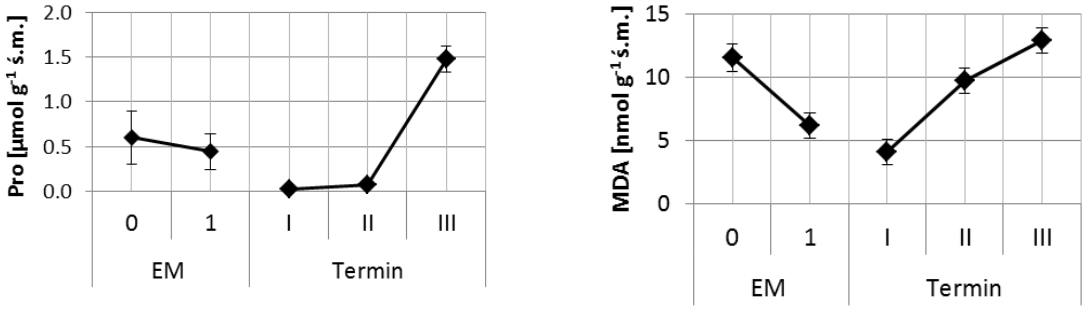
Praca miała na celu określenie wpływu Efektywnych Mikroorganizmów (EM) na poziom wolnej proliny i MDA, jako wskaźników biochemicznych adekwatnych w ocenie

aktywności biochemicznej i ogólnego stanu fizjologicznego badanych w różnych okresach wegetacji bazylii pospolitej (*Ocimum basilicum* L.).

Prolina jest enzymem, którego rolą jest m.in. osmoregulacja, stabilizacja błon komórkowych oraz ochrona tkanek rośliny przed degradacją. Jej poziom akumulacji w tkankach zależy od wieku rośliny, stadium rozwoju, a także od czynników abiotycznych, jak temperatura, usłonecznienie czy wilgotność. Szybki wzrost jej zawartości w roślinie obserwuje się w momencie zadziałania czynnika stresowego (Verbruggen i Hermans, 2008).

Analiza wyników wykazała istotny wpływ zastosowania EM i terminu pomiaru na zawartość wolnej proliny w materiale roślinnym bazylii. Należy jednak zaznaczyć, że uzyskane procentowe współczynniki wpływu obu tych czynników wykazały znacznie większy wpływ terminu przeprowadzenia pomiaru na zawartość wolnej proliny (P=92,8%) niż stosowanie EM (P=1,22%).

W pierwszym i drugim terminie pomiaru, niezależnie od zastosowania EM, rośliny charakteryzowały się bardzo niską zawartością proliny od 0 do 0,02 μmol g^{-1} ś.m. Najwyższe stężenie proliny odnotowano w trzecim terminie, przy czym w przypadku roślin traktowanych preparatem EM było ono istotne niższe (1,48 μmol g^{-1} ś.m.) niż u roślin kontrolnych (1,74 μmol g^{-1} ś.m.) (Ryc. 1).



Ryc. 1. Wpływ badanych czynników na średnią zawartość proliny oraz średnia zawartość MDA w zielu bazylii pospolitej.

Dialdehyd malonowy powstaje w wyniku bardzo istotnego zaburzenia w komórce jakim jest peroksydacja lipidów. Powoduje on zmiany struktury błony komórkowej, co prowadzi do jej dezintegralności i rozprzężenia fosforylacji w mitochondriach, działa również mutagennie na DNA (Islam i in., 2009).

Procentowy udział wpływu EM kształtował się na poziomie ok. 23%, przy czym ponownie najistotniejszy wpływ miał termin pomiaru 42,3%.

Najniższe stężenie MDA odnotowano na początku wegetacji, zarówno w przypadku roślin traktowanych EM (3,17 nmol g^{-1} ś.m), jak i kontrolnych (5,01 nmol g^{-1} ś.m.) (Fig. 1). W drugim terminie, niezależnie od wariantu doświadczenia, parametr ten kształtował się na podobnym poziomie, tj. 9,57 - 9,84 nmol g^{-1} ś.m. Najwyższe stężenie MDA odnotowano pod koniec wegetacji u roślin kontrolnych 20,22 nmol g^{-1} ś.m., było ono 4-krotnie wyższe w porównaniu do roślin traktowanych EM, u których odnotowano stężenie o wartości 5,65 nmol g^{-1} ś.m.

4.2. The influence of Effective Microorganisms and number of buds per cane in viticulture on chemical composition in fruits (P2)

W wyniku ocieplenia klimatu strefa upraw winorośli przesuwa się na północ. Problemem w tych warunkach może być niska jakość owoców. Właściwy dobór agrotechniki oraz środków wspomagających rozwój roślin stanowią podstawę uzyskania polonów o wysokiej jakości.

Efektywne Mikroorganizmy to naturalny preparat ochronny, który swoim działaniem ma łagodzić stres fizjologiczny u roślin wywołany różnymi abiotycznymi czynnikami. Cięcie winorośli i prowadzenie krzewów na określoną liczę pędów sprzyja zawiązywaniu się owoców o optymalnym rozmiarze, właściwym kolorze oraz odpowiedniej zawartości ekstraktu. Polifenole są jedną z najczęściej badanych grup związków biologicznie czynnych, ze względu na swoje właściwości prozdrowotne. Skład profilu polifenolowego winogron zależy od wielu czynników, m.in.: odmiany winorośli, stopnia dojrzałości owoców, warunków klimatycznych i glebowych oraz stanu fizjologicznego rośliny (Pantelić i in., 2016).

Celem przeprowadzonego doświadczenia było zbadanie wpływu EM oraz liczby pędów na zawartość ekstraktu cukrowego, kwasowości i profil polifenoli w owocach dwóch odmian winorośli Regent i Cabernet Cortis uprawianych w północno-zachodniej Polsce. Przeprowadzono także analizę interakcji między badanymi czynnikami i ich wpływu na zawartość poszczególnych grup polifenoli.

Wykazano brak istotnego wpływu przeprowadzonych zabiegów, tj. liczby pędów i stosowania EM na zawartość w owocach ekstraktu ogólnego oraz ich kwasowość ogólną. Średnia zawartość ekstraktu ogólnego w owocach obu odmian kształtowała się w przedziale 15,85 – 17,95 °Brix, a kwasowość ogólna od 5,25 do 6,40 g dm^{-3}.

W aspekcie przydatności technologicznej plonu, zawartość związków polifenolowych jest jednym z najważniejszych parametrów jakościowych winogron oraz win. Polifenole mają bezpośredni wpływ na cechy organoleptyczne win, takie jak smak, cierpkość, gorycz i kolor. W owocach winorośli oznaczono 36 związków polifenolowych należących do antocyjanów, kwasów fenolowych, flawonoli i flawan-3-oli. Wyniki zawartości poszczególnych grup polifenoli zebrano i przedstawiono w Tabeli 1.

W doświadczeniu wykazano istotny wpływ badanych czynników na całkowitą zawartość polifenoli w owocach. Najistotniejszym czynnikiem różnicującym zawartość polifenoli była odmiana (P=69,8%), następnie liczba pędów (P=10,8%) oraz zastosowanie EM (P=7,9%).

Tabela 1. Wpływ sposobu prowadzenia winorośli oraz stosowania EM na zawartość polifenoli ogółem w owocach winogron odmian Regent i Cabenet Cortis (test Tukey'a p=0,05).

Liczba pędów	'Regent'		**średnia**	'Cabernet Cortis'		**średnia**
	K	EM		K	EM	
Polifenole (mg 100 g^{-1} ś.m.)						
4	528,73^{a}	469,98^{ab}	**499,35^{A}**	382,42^{cd}	357,01^{cde}	**369,71^{C}**
8	471,28^{ab}	418,03^{bc}	**444,66^{B}**	342,57^{de}	317,29^{e}	**329,93^{D}**
średnia	**500,01A**	**444,00B**		**362,50C**	**337,15C**	
Antocyjany (mg 100 g^{-1} ś.m.)						
4	374,28^{a}	333,06^{bc}	**353,67^{A}**	206,38^{d}	207,50^{d}	**206,94^{C}**
8	338,81^{ab}	297,14^{c}	**317,98^{B}**	198,01^{d}	184,77^{d}	**191,39^{C}**
średnia	**356,55A**	**315,10B**		**202,20C**	**196,14C**	
Kwasy fenolowe (mg 100 g^{-1} ś.m.)						
4	23,13^{a}	22,18^{a}	**22,66^{C}**	36,37^{c}	33,14^{cd}	**34,76^{A}**
8	30,83^{bcd}	26,34^{ab}	**28,59^{B}**	29,02^{bc}	25,64^{ab}	**27,33^{B}**
średnia	**26,98BC**	**24,26C**		**32,69A**	**29,39AB**	
Flawanole (mg 100 g^{-1} ś.m.)						
4	24,33^{a}	15,62^{b}	**19,98^{A}**	13,73^{bc}	11,34^{c}	**12,54^{B}**
8	22,06^{a}	14,60^{bc}	**18,33^{A}**	12,28^{bc}	10,67^{c}	**11,47^{B}**
średnia	**23,20A**	**15,11B**		**13,00BC**	**11,01C**	
Flawon-3-ole (mg 100 g^{-1} ś.m.)						
4	106,99^{a}	99,11^{b}	**103,05^{A}**	125,94^{a}	105,02^{a}	**115,48^{A}**
8	79,58^{c}	79,94^{c}	**79,76^{C}**	103,27^{ab}	96,21^{b}	**99,74^{B}**
średnia	**93,28BC**	**89,53C**		**114,60A**	**100,62B**	

K – kontrola; EM – Efektywne Mikroorganizmy

Efektywne Mikroorganizmy miały znaczący wpływ na obniżenie zawartości polifenoli ogółem w owocach badanych odmian winogron. Najniższy poziom związków polifenolowych stwierdzono w owocach z roślin prowadzonych na 8 pędów i uprawianych z wykorzystaniem EM – 'Caberent Cortis' 317,29 mg 100 g^{-1} ś.m., 'Regent' 418,03 mg 100 g^{-1} ś.m.. Natomiast, najwyższą zawartością polifenoli charakteryzowały się owoce odmiany Regent, z roślin prowadzonych na 4 pędy uprawianych w warunkach kontrolnych (bez EM) 528,73 mg 100 g^{-1} ś.m.

Antocyjany są w głównej mierze odpowiedzialne za barwę owoców i pozyskiwanego z nich moszczu. W owocach obu odmian stanowiły one największą grupę związków polifenolowych – 'Regent' 71,2%, 'Cabernet Cortis' 56,9 %. Przeprowadzone zabiegi miały istotny wpływ na badany parametr głównie w przypadku odmiany Regent. Najwyższy procentowy udział wpływu czynnika na zawartość antocyjanów wykazano dla odmiany (P=87,9%). Natomiast udział pozostałych czynników, tj. EM i liczby pędów, wyniósł odpowiednio: P=1,5% i P=3,1%. Owoce odmiany Regent poddane działaniu EM charakteryzowały się istotnie mniejszą zawartością antocyjanów (315,10 mg 100 g^{-1} ś.m.) w porównaniu z kontrolą (356,55 mg 100 g^{-1} ś.m.) bez względu na sposób prowadzenia roślin. Natomiast owoce z roślin o 4 pędach miały istotnie większą zawartość antocyjanów (499,35 mg 100 g^{-1} ś.m.) niż z krzewów o 8-mu pędach (444,66 mg 100 g^{-1} ś.m.). U odmiany Cabernet Cortis nie stwierdzono istotnego wpływu przeprowadzonych zabiegów na wartości tego parametru. Natomiast, wykazano istotnie wyższą całkowitą średnią zawartość antocyjanów w owocach odmiany Regent (335,83 mg 100 g^{-1} ś.m.) niż w owocach odmiany Cabernet Cortis (199,17 mg 100 g^{-1} ś.m.).

Krzewy z różną liczbą pędów charakteryzują się odmiennym mikroklimatem korony. Stosowanie cięcia powoduje zmianę natężenia promieniowania słonecznego, temperatury czy wilgotności w koronie, co najczęściej ma istotny wpływ na skład i jakość winogron (Haselgrove i in., 2000).

Kwasy fenolowe stanowiły 5,4% 'Regent' i 8,9% 'Cabernet Cortis' całkowitej zawartości polifenoli. Średnia zwartość kwasów fenolowych u obu odmian była głównie determinowana liczbą pędów. Największy wpływ miała interakcja między odmianą a liczbą pędów (P=43,2%), następnie odmiana (P=28,5%) i stosowanie EM (P=8,8%). U odmiany Regent znacznie więcej kwasów fenolowych stwierdzono w owocach roślin z 8-ma pędami (28,59 mg 100 g^{-1} ś.m.) niż z 4-ma pędami (22,66 mg 100 g^{-1} ś.m.). Natomiast u odmiany Cabernet Cortis większą zawartość kwasów odnotowano w owocach z roślin z 4-ma pędami (34,76 mg 100 g^{-1} ś.m.) niż w tych z roślin z 8-ma pędami (27,33

mg 100 g^{-1} ś.m.). Odmiana Cabernet Cortis charakteryzowała się istotnie wyższą zawartością kwasów fenolowych (31,04 mg 100 g^{-1} ś.m.) w porównaniu do owoców odmiany Regent (25,62 mg 100 g^{-1} ś.m.).

Flawonole to pigmenty żółte, które w winach czerwonych są maskowane przez antocyjany, jednak wpływają na ich barwę przez koopigmentację, której efektem jest wzmocnienie ekstrakcji antocyjanów podczas produkcji wina. W badaniach grupa ta stanowiła 4,1% całkowitej zawartości polifenoli w przypadku odmiany Regent i 3,4% dla 'Cabernet Cortis'. Najbardziej istotnym czynnikiem, który kształtował zawartość flawonoli w owocach była ponownie odmiana winorośli (P=52,8%), następnie stosowanie EM (P=26,2%) oraz ich interakcja (P=9,6%). Stosowanie EM miało istotny wpływ na zawartość flawonoli w owocach odmiany Regent, niezależnie od ilości pędów. Owoce tej odmiany pochodzące z roślin traktowanych EM miały istotnie niższą zawartość flawonoli (15,11 mg 100 g^{-1} ś.m.) w porównaniu do roślin uprawianych bez EM (23,20 mg 100 g^{-1} ś.m.). W przypadku odmiany Cabernet Cortis nie wykazano istotnego wpływu przeprowadzonych zabiegów na badany parametr. Odmiana Regent charakteryzowała się większą zawartością flawonoli (19,15 mg 100 g^{-1} ś.m) niż odmiana Cabernet Cortis (12,01 mg 100 g^{-1} ś.m.).

Flawan-3-ole to grupa związków taninowych, które licznie występują głównie w winogronach odmian czerwonych. Odpowiadają one za 'strukturę' wina, cierpkość, gorycz. Odgrywają również bardzo ważną rolę w stabilizacji barwy czerwonej w winie (Guerrero i in. 2009). Całkowita zawartość flawan-3-oli u odmiany Regent wyniosła 91,4 mg 100 g^{-1} ś.m., a u odmiany Cabernet Cortis 107,61 mg 100 g^{-1} ś.m. Tym samym związki należące do tej grupy stanowiły odpowiednio 19,4% i 30,8% całkowitej zawartości oznaczonych polifenoli w owocach badanych odmian winorośli. Znaczący wpływ na całkowitą zawartość flawan-3-oli miała odmiana (P=28,6%), liczba pędów (P=41,4%), EM (P= 8,6).

W owocach obu odmian winorośli prowadzonych na większą liczbę pędów odnotowano znacząco niższą średnią zawartość flawan-3-oli w porównaniu do owoców z krzewów prowadzonych na mniejszą liczbę pędów. Zastosowanie EM spowodowało istotny obniżenie zawartości flawan-3-oli u odmiany Cabernet Cortis (114,60 mg 100 g^{-1} ś.m.; kontrola 100,62 mg 100 g^{-1} ś.m).

4.3. Effect of Tytanit® on the physiological activity of wild strawberry (*Fragaria vesca* L.) grown in salinity conditions (P3)

Niedobór zasobów słodkiej wody, zanieczyszczenie środowiska oraz zwiększenie zasolenia gleby i wody stanowią realny problem w skali globalnej. Szacuje się, że na całym świecie 20% całkowitej uprawy i 33% nawadnianych gruntów rolnych jest dotkniętych wysokim zasoleniem (Shrivastava i Kumar, 2015). Wpływa to na zmniejszenie powierzchni uprawnej oraz pogorszenie wydajności i jakości plonów. Stosowanie preparatów wspomagających wzrost roślin w takich warunkach staje się nieodzowne. Poziomka pospolita (*Fragaria vesca* L.) jest gatunkiem niszowym. Ze względu na intensywny smak i aromat oraz wysoką zawartość antyoksydantów jej owoce są cenione i poszukiwane zarówno przez rynek świeżych owoców, przemysł przetwórczy, cukierniczy, jak i również kosmetyczny (Yurdugul, 2008).

Celem przeprowadzonego doświadczenia było zbadanie wpływu oraz skuteczności działania preparatu Tytanit® na aktywność fizjologiczną poziomki pospolitej odmiana Baron von Solemacher uprawianej w warunkach zasolenia.

W doświadczeniu wykazano, iż największy współczynnik wpływu na zawartość barwników asymilacyjnych, tj.: chlorofilu *a* i *b* oraz karotenoidów miało zasolenie, od 27,40% do 32,07%. Dla chl. *a* i karotenoidów drugi co do istotności wpływ miała interakcja między zasoleniem a terminem, odpowiednio P=24,17% i P=29,27%, a dla chl. *b* interakcja między zastosowaniem Tytanitu, a terminem P=20,94%, natomiast wpływ zastosowania Tytanitu wyniósł P=12,27%.

W pierwszym terminie pomiaru, niezależnie od poziomu zasolenia podłoża, nie odnotowano znaczącego wpływu Tytanitu na zawartość poszczególnych barwników asymilacyjnych. Natomiast w drugim terminie, rośliny poddane działaniu preparatu charakteryzowały się istotnie niższą zawartością chlorofilu *a*, *b* oraz karotenoidów, (odpowiednio 1,359 mg g^{-1}ś.m.; 0,573 mg g^{-1}ś.m.; i 3,068 mg g^{-1} ś.m.), w porównaniu do roślin uprawianych bez Tytanit® (chl *a* 1,797 mg g^{-1} ś.m.; chl *b* 0,788 mg g^{-1} ś.m.; karotenoidy 3,965 mg g^{-1} ś.m.) (Tabela 2).

W przeprowadzonych badaniach wykazano wpływ wszystkich badanych czynników na zawartość proliny w liściach poziomki. Przy czym najistotniejszy wpływ miało zasolenie P=56,84%, termin P=28,93%, a najmniejszy zastosowanie Tytanitu P=0,53%.

Tabela 2. Wpływ Tytanitu na zawartość barwników asymilacyjnych oraz stężenie wolnej proliny w liściach poziomki pospolitej uprawianej na podłożach o różnym poziomie zasolenia (test Tukey'a p=0,05).

Zasolenie	BBCH15		**średnia**	BBCH60		**średnia**
	0	T		0	T	
			Chlorofil *a* (mg g^{-1} ś.m.)			
S1	$1{,}651^{bcd}$	$1{,}921^{ab}$	**1,786^{B}**	$2{,}007^{ab}$	$2{,}185^{a}$	**2,096^{A}**
S2	$1{,}777^{bc}$	$1{,}736^{bcd}$	**1,756^{B}**	$1{,}852^{abc}$	$1{,}396^{d}$	**1,624^{B}**
S3	$1{,}758^{bcd}$	$1{,}663^{bcd}$	**1,710^{B}**	$1{,}535^{cd}$	$0{,}497^{e}$	**1,016^{C}**
średnia	**1,729A**	**1,773A**		**1,797A**	**1,359B**	
			Chlorofil *b* (mg g^{-1} ś.m.)			
S1	$0{,}605^{c}$	$0{,}700^{abc}$	**0,653^{B}**	$0{,}836^{a}$	$0{,}783^{ab}$	**0,810^{A}**
S2	$0{,}666^{bc}$	$0{,}695^{abc}$	**0,681^{B}**	$0{,}780^{ab}$	$0{,}611^{c}$	**0,695^{B}**
S3	$0{,}699^{abc}$	$0{,}661^{bc}$	**0,680^{B}**	$0{,}748^{abc}$	$0{,}326^{d}$	**0,537^{C}**
średnia	**0,657B**	**0,685B**		**0,788A**	**0,573C**	
			Karotenoidy (mg g^{-1} ś.m.)			
S1	$3{,}445^{cde}$	$4{,}008^{bcd}$	**3,727^{B}**	$4{,}387^{ab}$	$5{,}014^{a}$	**4,700^{A}**
S2	$3{,}720^{bcde}$	$3{,}777^{bcde}$	**3,740^{B}**	$4{,}219^{abc}$	$3{,}078^{e}$	**3,650^{B}**
S3	$3{,}836^{bcde}$	$3{,}564^{bcde}$	**3,700^{B}**	$3{,}289^{de}$	$1{,}111^{f}$	**2,200^{C}**
średnia	**3,667A**	**3,783A**		**3,965A**	**3,068B**	
			Prolina (µmol g^{-1} ś.m.)			
S1	$0{,}585^{f}$	$0{,}283^{f}$	**0,434^{D}**	$1{,}048^{e}$	$1{,}108^{e}$	**1,078^{C}**
S2	$0{,}445^{f}$	$0{,}527^{f}$	**0,486^{D}**	$1{,}415^{de}$	$1{,}752^{cd}$	**1,583^{B}**
S3	$2{,}100^{c}$	$1{,}365^{de}$	**1,733^{B}**	$4{,}905^{a}$	$4{,}229^{b}$	**4,567^{A}**
średnia	**1,044B**	**0,725C**		**2,456A**	**2,363A**	

S1 –podłoże o zasoleniu 32.5 mM L^{-1}; S2 - 50 mM L^{-1}; S3 – 100 mM L^{-1}; 0 – brak Tytanitu; T – Tytanit®

Wykazano, że wyższe zasolenie podłoża (50 i 100 mM l^{-1}), niezależnie od terminu pomiaru, istotnie zwiększyło ilości proliny w liściach poziomki. Natomiast stosowanie preparatu istotnie obniżyło stężenie proliny tylko w przypadku młodych roślin (Tytanit® 0,725 nmol g^{-1} ś.m.; bez Tytanitu 1,044 nmol g^{-1} ś.m).

W badaniach wykazano znaczący wpływ zasolenia i terminu pomiaru na obniżenie względnej zawartości wody w liściach poziomki. Zasolenie miało wyraźnie większy wpływ niż termin. Natomiast Tytanit® różnicował wielkość wskaźników RWC i WSD w badanych terminach, ale w sposób nieistotny.

Pomiar fluorescencji chlorofilu znajduje zastosowanie w badaniach ekofizjologicznych, monitorowaniu upraw i ekosystemów zagrożonych czynnikami

fitotoksycznymi oraz w badaniach tolerancji roślin na różnorodne czynniki stresowe. Jest wysoce czułą próbą reakcji fotosyntetycznych roślin, która pozwala na wczesne wykrywanie zmian w ogólnym statusie rośliny (Kalaji i Łaboda, 2010).

W przeprowadzonym doświadczeniu z poziomką pospolitą maksymalna potencjalna fotochemiczna wydajność fotosystemu PSII (Fv/F_M) była uzależniona w największym stopniu od terminu dokonania pomiaru P=37,28%, następnie od interakcji między zasoleniem a terminem P=32,04%, i od samego zasolenia P=25,09%.

Maksymalna fotochemiczna wydajność PSII (Fv/F_M) to wiarygodny wskaźnik aktywności fotochemicznej aparatu fotosyntetycznego. Dla większości roślin w fazie pełnego rozwoju i w warunkach optymalnych wartość tego parametru wynosi ok. 0,83 (Kalaji i Łaboda 2010). W doświadczeniu własnym z poziomką średnia wartość Fv/F_M dla roślin w stadium rozwoju BBCH15, wynosiła ok. 0,74 (Tabela 3). Starsze rośliny (BBCH60) charakteryzowały się istotnie niższą wartością Fv/F_M w porównaniu do młodszych, jednak, należy tu wskazać, że starsze rośliny traktowane Tytanitem miały istotnie wyższy wskaźnik Fv/F_M (ok. 0,51) niż rośliny uprawiane bez Tytanitu (ok. 0,44). Obniżenie wartości tej cechy świadczy o działaniu czynnika stresowego, który uszkodził funkcje PSII, zmniejszając efektywność transportu elektronów.

Tabela 3. Wpływ Tytanitu na wydajność aparatu fotosyntetycznego poziomki pospolitej uprawianej na podłożach o różnym poziomie zasolenia (test Tukey'a p=0,05).

Zasolenie	BBCH15		**średnia**	BBCH60		**średnia**
	0	T		0	T	
			Fv/Fm			
S1	0,750^{a}	0,721^{a}	**0,735^{A}**	0,691^{a}	0,667^{a}	**0,679^{A}**
S2	0,740^{a}	0,723^{a}	**0,731^{A}**	0,643^{a}	0,723^{a}	**0,683^{A}**
S3	0,716^{a}	0,699^{a}	**0,708^{A}**	0,006^{b}	0,025^{b}	**0,014^{B}**
średnia	**0,735A**	**0,714A**		**0,436C**	**0,505B**	
			Fv/Fo			
S1	3,012^{a}	2,785^{a}	**2,898^{A}**	2,500^{a}	2,157^{a}	**2,328^{A}**
S2	2,944^{a}	2,645^{a}	**2,794^{A}**	1,985^{a}	2,669^{a}	**2,327^{A}**
S3	2,650^{a}	2,562^{a}	**2,606^{A}**	0,006^{b}	0,027^{b}	**0,015^{B}**
średnia	**2,868A**	**2,664A**		**1,453B**	**1,743B**	

S1 –podłoże o zasoleniu 32.5 mM L^{-1}; S2 - 50 mM L^{-1}; S3 – 100 mM L^{-1}; 0 – brak Tytanitu; T – Tytanit®

Na maksymalną efektywność rozszczepienia wody po donorowej stronie PSII (Fv/Fo) najistotniejszy wpływ miało zasolenie P=26,54%, mniej istotny termin pomiaru P=25,88%, a najmniejszy interakcja między tymi czynnikami P=17,11%. Wartość błędu (P=28,21%) podobnie jak w przypadku asymilacji dwutlenku węgla przewyższała udział badanych czynników; stosowania Tytanitu, terminu i zasolenia. Wskazuje to na większy wpływ niebadanych w doświadczeniu czynników na wartość Fv/Fo u poziomki. Średnia wartość tego parametru była istotnie niższa w drugim terminie pomiaru niezależnie od stosowania Tytanitu. Najwyższe zasolenie podłoża spowodowało istotne obniżenie parametru w drugim terminie, średnia wartość Fv/Fo była niemal dwustu krotnie niższa od średniej wartości tego parametru mierzonego w pierwszym terminie.

W roślinach rosnących w warunkach zasolenia następuje zmniejszenie wartości stosunku Fv/Fo, co wskazuje na obniżenie wydajności reakcji rozszczepienia wody i osłabienie fotosyntetycznego transportu elektronów. Niższa wartość Fv/Fo odnotowana u roślin traktowanych Tytanitem w pierwszym terminie w porównaniu do kontroli może świadczyć o negatywnym działaniu preparatu na fazę jasną fotosyntezy. Obniżenie współczynnika Fv/Fo jest wskaźnikiem uszkodzeń strukturalnych, które występują w tylakoidach i wpływa na fotosyntetyczny transport elektronów.

4.4. Influence of Tytanit® and EM on biochemical, physiological, and qualitative parameters of common bean (P4)

Zwiększone występowanie stresów abiotycznych powodowane zmianami klimatycznymi wpływa na jakość i wielkość plonów roślin uprawnych. Rolą biostymulatorów w głównej mierze jest niwelowanie szkodliwego działania różnych czynników stresowych na rośliny i zapewnienie wysokich plonów o dobrej jakości (Higa, 2004). Fasola zwyczajna (*Phasoleus vulgaris* L.) jest jedną z najważniejszych gospodarczo roślin uprawnych na świecie.

Podjęte badania miały na celu ocenę wpływu Efektywnych Mikroorganizmów oraz Tytanitu na aktywność fizjologiczną i biochemiczną oraz cechy jakościowe fasoli zwyczajnej w okresie jej wegetacji.

Wykazano istotny wpływ badanych czynników na zawartość barwników asymilacyjnych w liściach fasoli. Największy wpływ na zawartość chl *a* i chl *b* oraz karotenoidów miał termin wykonania pomiaru, odpowiednio P=37,20%, P=42,32%, P =42,95%. Interakcja między zastosowanymi preparatami a terminem miała istotny wpływ na zawartość chl *a* oraz karotenoidów (P=31,50% i P=22,78%). Mniejszy, ale

również istotny wpływ miało zastosowanie preparatów (P=7,62% i P=11,75%). Rośliny traktowane preparatami charakteryzowały się istotnie wyższą średnią zawartością badanych barwników niż kontrola, przy czym najwyższą zawartość miały rośliny uprawiane z wykorzystaniem Tytanitu: chl *a* 1,757 mg g^{-1} ś.m., chl *b* 0,707 mg g^{-1} ś.m. i karotenoidy 0,956 mg g^{-1} ś.m. (Tabela 4).

Tabela 4. Wpływ Tytanitu oraz EM na zawartość barwników asymilacyjnych, stężenie wolnej proliny i MDA w liściach fasoli zwyczajnej(test Tukey'a p=0,05).

Preparat	Faza fenologiczna			**średnia**
	15 BBCH	24 BBCH	65 BBCH	
Chlorofil a (mg g^{-1} ś.m.)				
K	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}**
średnia	**1,742A**	**1,456B**	**1,800A**	
Chlorofil b (mg g^{-1} ś.m.)				
K	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}**
średnia	**0,733A**	**0,590B**	**0,698A**	
Karotenoidy (mg g^{-1} ś.m.)				
K	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}**
średnia	**0,945A**	**0,745B**	**0,997A**	
MDA (nmol g^{-1} ś.m.)				
K	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}**
średnia	**40,93A**	**21,83B**	**19,56C**	
Prolina (µmol g^{-1} ś.m.)				
K	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}**
średnia	**0,201B**	**0,174B**	**0,707A**	

K – kontrola; T - Tytanit®; EM –Efektywne Mikroorganizmy

W przypadku roślin kontrolnych zawartość badanych barwników była na podobnym poziomie we wszystkich trzech terminach pomiaru. Natomiast u roślin

poddanych działaniu preparatów odnotowano istotnie wyższą zawartość barwników w pierwszym i trzecim terminie pomiaru w porównaniu do kontroli, a w drugim terminie zaobserwowano ich istotne obniżenie. Wiele cech roślin podlega dużej zmienności fenotypowej w zależności od działania różnych czynników środowiska i genotypu. Wysoka zawartość chlorofilu w fazie 3-go pędu może świadczyć o intensywnie zachodzącej morfogenezie, natomiast niższy poziom barwnika w fazie kwitnienia może wskazywać na spowolnienie tego procesu. Wzrost zawartości badanych barwników w przypadku roślin traktowanych Tytanitem i EM w okresie formowania strąków najprawdopodobniej jest wynikiem większego zapotrzebowania roślin na asymilaty alokowane w tworzących się strąkach i nasionach, a z tym związany wzrost intensywności procesu asymilacji i bezpośrednio wpływających na ten proces barwników fotosyntetycznych. Tytanit® i EM okazały się aktywujące syntezę barwników.

Wykazano istotny wpływ zastosowanych preparatów oraz terminu pomiaru na zawartość proliny w tkance roślinnej. Przy czym najistotniejszy wpływ miał termin pomiaru P=95,58%, a najmniejszy zastosowanie preparatów P=1,25%.

Największą średnią zawartość proliny miały rośliny traktowane Tytanitem i kontrola odpowiednio 0,378 i 0,382 μmol g^{-1} ś.m., istotnie mniejszą zawartość miały rośliny traktowane preparatem EM 0,321 μmol g^{-1} ś.m. Najistotniejszy wzrost średniej zawartości proliny w świeżej tkance roślinnej odnotowano w trzecim terminie pomiaru dla wszystkich badanych wariantów. Przy czym rośliny traktowane Tytanitem charakteryzowały się najwyższym poziomem proliny ze wszystkich badanych wariantów badanych w tym terminie (0,756 μmol g^{-1} ś.m.). Mniejsza zawartość proliny u roślin traktowanych EM może świadczyć o łagodzącym wpływie tego preparatu na reakcje stresowe, które są wynikiem działania różnych czynników stresowych pojawiających się podczas ontogenezy czy też samym procesem starzenia się. Natomiast najwyższa średnia zawartość proliny w wariancie z Tytanitem sugeruje działanie preparatu podobne do abiotycznego czynnika stresowego, pobudzającego syntezę proliny.

Podobnie jak w przypadku proliny, wykazano, że na zawartość dialdehydu malonowego (MDA) w świeżej tkance roślinnej miały wpływ wszystkie badane czynniki. Przy czym, najistotniejszy wpływ miał termin wykonania pomiaru P=97,24%, a najmniejszy zastosowanie preparatów P=0,20%. Rośliny uprawiane z zastosowaniem preparatów charakteryzowały się wyższą średnią zawartością MDA niż kontrola, przy czym istotnie wyższą zawartość miały rośliny traktowane Tytanitem 27,97 nmol g^{-1} ś.m.. W pierwszym terminie pomiaru, traktowane rośliny cechowała istotnie wyższa zawartość

MDA (EM 43,22 nmol g^{-1} ś.m., Tytanit® 41,33 nmol g^{-1} ś.m.) niż w kontroli (38,22 nmol g^{-1} ś.m.). Natomiast w trzecim terminie oba preparaty istotnie obniżyły poziom MDA w porównaniu do kontroli. Wysokie stężenie MDA w fazie 15 BBCH wskazuje na wystąpienie czynnika stresowego. Natomiast zaobserwowana wysoka średnia zawartość MDA w fasoli traktowanej tymi preparatami w porównaniu do kontroli może świadczyć o niekorzystnym ich działaniu na ten gatunek i wywołaniu stresu oksydacyjnego.

W strąkach fasoli oznaczono zawartość siedmiu pierwiastków: Na, Ca, K, Fe, Mn, Mg, P. Preparaty istotnie obniżyły zawartość Ca, Mn, Mg i P. Nie wykazano wpływu obu preparatów na zawartość potasu w strąkach fasoli. W przypadku Tytanitu odnotowano również znaczące obniżenie zawartości Na, a w przypadku EM wysoce istotne obniżenie zawartości Fe w porównaniu z kontrolą i Tytanitem (Tabela 5).

Tabela 5. Wpływ biostymulatorów na zawartość wybranych mikro i makroelementów w suchej masie strąków fasoli zwyczajnej (test Tukey'a p=0,05).

	Pierwiastki (g kg^{-1} s.m.)						
	Na	Ca	K	Fe	Mn	Mg	P
K	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]

K – kontrola; EM – Efektywne Mikroorganizmy; T - Tytanit®

Analiza statystyczna nie wykazała istotnego wpływu przeprowadzonych zabiegów na liczbę strąków oraz ich świeżą i suchą masę (Tabela 6). Przy czym, warto zaznaczyć, że wartości badanych parametrów były najwyższe w przypadku plonu z roślin kontrolnych (średnia liczba strąków zebranych z jednej rośliny 12,75; średnia świeża masa zebranych strąków z jednej rośliny 110,72 g; średnia sucha masa zebranych strąków z jednej rośliny 11,25 g).

Tabela 6. Uśrednione wyniki wybranych cech plonu fasoli zwyczajnej (test Tukey'a p=0,05).

	Liczba strąków z jednej rośliny		**Świeża masa strąków (g) z jednej rośliny**		**Sucha masa strąków (g) z jednej rośliny**	
	średnia	SD	średnia	SD	średnia	SD
K	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

K – kontrola; EM – Efektywne Mikroorganizmy; T - Tytanit®; SD – odchylenie standardowe

5. Podsumowanie i wnioski

Wyniki przedstawione w publikacjach P1 – P4 konfrontując z wynikami innych autorów wskazują na niejednoznaczne działanie preparatów EM oraz Tytanit® w wybranych uprawach roślin ogrodniczych. Ich skuteczność wydaje się być zależna od wielu czynników m.in.: warunków uprawy, doboru gatunku, a nawet odmiany. Precyzyjne określenie czynników determinujących skuteczność działania danego preparatu w danej uprawie jest konieczne w celu optymalizacji i racjonalizacji stosowania środków ochrony roślin oraz nawozów. Wyniki przedstawionych prac są uzupełnieniem wiedzy w zakresie wykorzystania preparatów EM oraz Tytanit® w uprawach roślin ogrodniczych, a na ich podstawie sformułowano następujące wnioski:

1. Efektywne Mikroorganizmy łagodziły skutki stresu oksydacyjnego poprzez obniżenie stężenia wolnej proliny oraz istotne zredukowanie procesu peroksydacji lipidów (obniżenie poziomu MDA) w zielu bazylii i u fasoli zwyczajnej. Wskazuje to na przydatność preparatu w aklimatyzacji tych roślin do warunków stresowych.

2. Tytanit® spowodował znaczne obniżenie poziomu proliny w młodszych roślinach poziomki pospolitej uprawianej w warunkach zasolenia, co może wskazywać na łagodzenie przebiegu reakcji stresowej na tym etapie rozwoju, wywołanej zasoleniem. Natomiast nie stwierdzono takiego wpływu preparatu w późniejszej fazie rozwoju tych roślin.

3. Tytanit® zastosowany w uprawiane poziomki pospolitej w warunkach wysokiego zasolenia podłoża znacząco zredukował zawartości barwników asymilacyjnych oraz istotne obniżył parametry wydajności aparatu fotosyntetycznego Fv/F_M i Fv/Fo w późniejszej fazie fenologicznej roślin, co może świadczyć o fitotoksyczności preparatu.

4. Zastosowanie Tytanitu, niezależnie od stopnia zasolenia podłoża, nie miało wpływu na poprawę bilansu wodnego (RWC) poziomki pospolitej, decydującego o kondycji fizjologicznej i produktywności roślin.

5. Zastosowanie Tytanitu, jak i Efektywnych Mikroorganizmów w uprawie fasoli zwyczajnej nie wpłynęło na wysokość plonu, natomiast znacząco obniżyło jego wartość biologiczną poprzez istotne zmniejszenie zawartości cennych żywieniowo pierwiastków

mineralnych w strąkach fasoli, takich jak: mangan, magnez, fosfor i wapń, oraz sodu (EM) i żelaza (Tytanit®).

6. Efektywne Mikroorganizmy obniżyły w owocach badanych odmian winorośli całkowitą zawartość polifenoli, związków o cennych właściwościach antyoksydacyjnych oraz nie miały wpływu na zawartość w owocach ekstraktu ogólnego i ich kwasowość ogólną, powodując tym samym obniżenie ich wartości biologicznej.

Na podstawie wykazanej w doświadczeniach bardzo zróżnicowanej reakcji fizjologicznej i biochemicznej badanych gatunków roślin ogrodniczych na stosowanie w ich uprawie preparatów EM oraz Tytanit® nie można potwierdzić założonej hipotezy badawczej, że preparaty te znacząco poprawiają kondycję fizjologiczną roślin ogrodniczych i wpływają na zwiększenie plonu oraz jego wartości biologicznej. Stosowanie EM oraz Tytanit® w uprawie przetestowanych roślin ogrodniczych celem polepszenia ich cech użytkowych wydaje się być nieuzasadnione.

Należy mieć na uwadze, że dobór odpowiednich do danej uprawy preparatów wspomagających rozwój i plonowanie roślin jest uzależniony od wielu czynników m.in.: gatunku, odmiany, fazy rozwojowej roślin, stopnia zasolenia podłoża, sposobu prowadzenia roślin. Dlatego wskazane jest wykonanie dalszych badań z zakresu biologii molekularnej oraz genetyki roślin. Pozwali to na lepsze zrozumienie i wyjaśnienie mechanizmów działania tych preparatów na poszczególne gatunki roślin ogrodniczych.

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EFFECT OF EFFECTIVE MICRO-ORGANISMS ON THE PROLINE AND MDA CONTENTS IN HERB PLANT MATERIAL OF *OCIMUM BASILICUM* L. VAR. PICCOLINO

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ABSTRACT

Research on effective micro-organisms (EM) mainly focuses on their effect on crop yield and crop quality. On the other hand, knowledge on the effect of EM on metabolic processes that take place in plants at the cellular level is not sufficiently systematised. The subject of this study was to evaluate the effect of an EM preparation on two oxidative stress parameters, i.e. free proline and malondialdehyde (MDA) contents in the green parts of sweet basil *Ocimum basilicum* L. var. Piccolino grown in pots. The concentration of free proline was determined by the ninhydrin reaction and the malondialdehyde concentration based on the reaction with thiobarbituric acid. Analysis of variance for selected factors showed a significant effect of interaction both EM and time on the decrease of proline and MDA concentration. Effect of singular factor i.e. EM has shown a favourable influence of the preparation on the oxidative stress parameters in sweet basil by lowering the concentration of proline and significant slowing down the process of lipid peroxidation in the plant tissues. EM can be used in crop growing as a preparation to facilitate the adaptation of plants to changing climatic and habitat conditions.

KEYWORDS:
biochemical parameters, cultivation, oxidative stress reduction, sweet basil

INTRODUCTION

Due to the progressing environmental degradation as a result of crop chemization, the organic farming, natural fertilisers and preparations are becoming more popular. Effective microorganism (EM) technology consisting biological preparations composed of specially selected, naturally occurring microorganisms, is one of the alternative for modern agriculture[1, 2].

Various environmental factors cause changes in plants metabolism [3,4]. Prolonged or increased exposure to stress factor results in an imbalance between the generation of reactive oxygen species (ROS) and its antioxidant abilities, what in consequences can lead to plants death [5,6]. Proline and malondialdehyde (MDA) are good indicators of oxidative stress in plants [7-9].

Proline participates in the stabilisation of proteins and cell membranes [10,11]. It also serves as an osmoprotectant and is a reservoir of nutrients for plants [9]. MDA induces changes in the structure of the cell membrane leading to its disintegration and uncoupling of phosphorylation in the mitochondria [12]. Its concentration depends on the level of ROS in tissues – the greater the production of free radicals, the higher the concentration of malondialdehyde [13].

Scientific reports to date mainly focus on the evaluation of the effect of EM on crop yield and crop quality [e.g. 14-17] but do not explore the issues that concern the effectiveness of their protective properties based on the metabolic mechanisms taking place in plants at the cellular level. Most of this type research is conducted on crop plants. On the other hand, there are only few reports on herbaceous plants that enjoy the growing interest in Poland due to their high biological value. A very valuable herbaceous plant, of a wide range of application, is sweet basil (*Ocimum basilicum* L.) and its new varieties.

Therefore, the study determines the effect of EM on the level of free proline and MDA, the biochemical indicators adequate in the evaluation of biochemical activity and general physiological condition of sweet basil var. Picollino.

MATERIAL AND METHODS

Material. In 2014-2015, during the growing season, a two-year pot experiment with sweet basil (*Ocimum basilicum* L.) var. Piccolino was conducted. The plant material came from a private horticultural farm in Szczecin. Biochemical analyses on the acquired material were performed at the laboratory of the Department of Plant Physiology and Biochemistry, Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology in Szczecin.

The experimental material was sweet basil var. Piccolino and EM. Sweet basil is an annual plant of the family *Lamiaceae*; the variety being tested is characterised by small leaves and a high content of essential oils. EM are natural preparations to support plant growth, being widely used in organic farming. They are composed of: lactic acid bacteria (*Lactobacillus casei*), photosynthetic bacteria (*Rhodopseudomonas palustrus*), yeasts (*Saccharomyces albus*), actinomycetes (*Streptomyces albus*) and mould (*filamentous*) fungi *(Aspergillus oryzae*).

A two-factor pot experiment was set up following the randomised complete block design in three replications. The first factor was 2 levels of EM application (level 1 involved the use of EM in cultivation, while level 2 is a control, without EM). The second factor was times of measurement (3 levels).

Sweet basil seeds, in the amount of 10 seeds per pot, were sown into a ready-made peat-based substrate with pH 5.5-6.5, salinity of 1.9 g $NaCl \cdot dm^{-3}$ and with a starter dose of NPK compound fertiliser 14-16-18 in the amount of 0.6 $kg \cdot m^{-3}$.

From the moment the seeds were sown, the objects intended for the application of effective micro-organisms were watered with an aqueous EM solution at a 1:100 dilution, every 7 days, in accordance to the manufacturer's recommendations. On other days, the plants were watered without addition of the EM preparation. On the other hand, the objects not intended for EM application were watered with plain water only.

The plant material for analyses was collected three times at monthly intervals, i.e. at the beginning of June, July and August. On all the dates, free proline and MDA contents in fresh herb parts of sweet basil were determined.

Proline (Pro) determination. The concentration of free proline in fresh green parts of sweet basil was determined by the ninhydrin reaction according to the method developed by Bates et al. [18].

Approximately 0.5 g of fresh plant tissue was homogenised in the presence of 3% aqueous solution of salicylic acid (10 ml), and the resultant homogenate was filtered through a filter paper. To the upper aqueous phase, 2 ml of acidic ninhydrin and 2 ml of glacial acetic acid were added. Next, the resultant solution was mixed thoroughly and, after pouring it into the closed tubes, placed in an incubator set at 90-100°C. After 1 hour, the tubes were transferred into an ice bath for 15 minutes to cool them. Then, 4 ml of toluene was added to each tube and they were shaken for 30 minutes. The samples prepared this way were left to allow the phases to separate. The upper phase (toluene) was sampled to determine the absorbance of chromatophore, against the blank, at the wavelength $\lambda = 520$ nm.

Malondialdehyde (MDA) determination. The concentration of malondialdehyde was determined by a slightly modified method according to Sudhakar et al. [19] that is based on the reaction of MDA with thiobarbituric acid.

The acquired plant material (1g) was homogenised with 0.1% TCA, then the resultant homogenate was filtered. To 1 cm^3 of the supernatant, 4 cm^3 of 0.5% TBA (in 20% TCA) was added. The closed tubes were placed in a water bath at 90-100°C and shaken for 30 minutes. Next, the tubes were placed in an ice bath for 15 minutes to cool them. The samples prepared this way were filtered once again, and then the absorbance against the reagent blank was determined in them at the wavelengths $\lambda = 532$ nm and $\lambda = 600$ nm. After removing the nonspecific turbidity being measured at $\lambda = 600$ nm, the MDA concentration was calculated using the mili-molar absorbance coefficient 155 $mM^{-1} \cdot cm^{-1}$.

Both determinations were made using a Shimadzu 1800 UV-Vis spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, Md., USA).

Statistics. The findings with regard to the effect of effective microorganisms on proline and malondialdehyde concentrations in the plant material were subjected to a two-way analysis of variance (ANOVA). Homogeneous groups were determined by the Tukey's test at the significance level $\alpha = 0.05$.

RESULTS

A significant effect of EM application on the concentration of free proline in the analysed plant material was shown (Tab. 1). The analysis of variance showed that the most significant statistical factor affecting the proline level in sweet basil herb was the time of taking measurements – 92.8 % (Tab. 1). The interaction of both factors, i.e. EM and time, had a significant effect on the analysed parameter – 4.7%.

Figures 1 and 2 present the effect of individual factors on the proline content. Under control conditions (without EM), the proline content was slightly higher than after EM application, the difference being however not significant. On the other hand, significant differences were found in the proline content depending on the time of measurement. The lowest proline concentration, ranging from 0 to 0.02 $\mu mol \cdot g^{-1}$ f.w., was determined on the first and the second date of taking measurements for the two experimental variants. Its highest concentration was observed on the third date of taking measurements, regardless of the EM level, i.e. 1.48 $\mu mol \cdot g^{-1}$ f.w. (Fig. 1).

TABLE 1
Analysis of variance for selected factors and interaction between the factors affecting the proline content.

Factor / interaction	SS	Df	MS	F	p	X
EM	0.11	1	107	11.45	0.005	1.22
Time	8.15	2	4074	436.13	0.000	92.80
EM x Time	0.41	2	207	22.11	0.000	4.70
Error	0.11	12	9			1.28

SS – sum of squared deviations from the mean, Df – degrees of freedom, MS – mean square (MS=SS/Df), F – F-test value, p – probability of error, X – percent effect of factors on the analysed property.

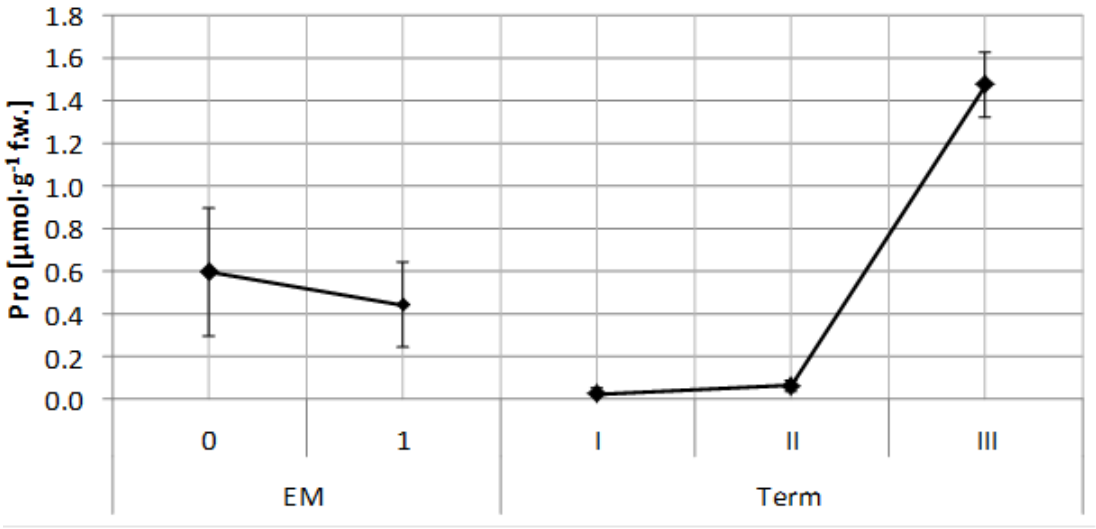

FIGURE 1
Average Pro concentrations in sweet basil herb for individual experimental factors.

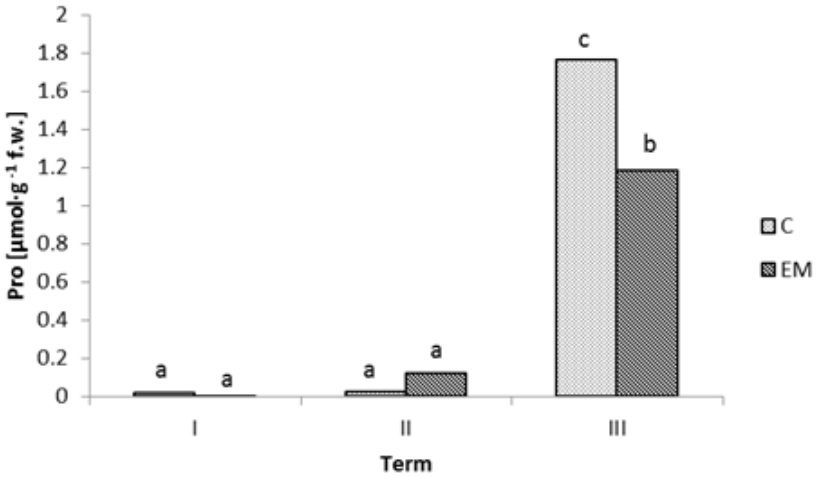

FIGURE 2
Pro content [µmol×g^{-1} f.w.] in the green parts of EM-treated sweet basil and under control conditions (without EM) depending on the time of measurement.

TABLE 2
Analysis of variance for selected factors and interaction between the factors affecting the MDA content.

Factor / interaction	SS	Df	MS	F	p	X
EM	130.07	1	130.073	361.45	0.000	22.89
Time	240.38	2	120.189	333.98	0.000	42.30
EM x Time	193.47	2	96.735	268.81	0.000	34.05
Error	4.32	12	0.360			0.76

SS – sum of squared deviations from the mean, Df – degrees of freedom, MS – mean square (MS=SS/Df), F – F-test value, p – probability of error, X – percent effect of factors.

Also on the third date of taking measurements – at the end of the growing season, a significant difference was found between the control plants (1.74 µmol·g^{-1} f.w.) and those with EM added (1.18 µmol·g^{-1} f.w.) (Fig. 2.). The EM addition decreased the proline content.

The EM preparation applied in the experiment also significantly decreased the malondialdehyde concentrations in the analysed plant material (Tab. 2.). The percent effect of this factor amounted to around 23%, with the time of taking measurements having once again the most significant effect on the MDA content in the plant tissue – 42.3%. The interaction of the two factors also showed a significant effect on the analysed parameter – around 34%.

Figures 3 and 4 present the effect of individual factors on the MDA content in the analysed material. The application of EM resulted in a 2-fold decrease in the MDA content in sweet basil herb compared to the control (Fig. 3). The time of taking measurements also significantly affected the average proline content in the analysed material. On the 1st date, its content was the lowest, whereas on the 3rd one the highest.

A significant interaction between the analysed experimental factors was observed (Fig. 4). The lowest MDA concentration was found in the plants collected on the 1st date and after EM application (3.17 nmol·g^{-1} f.w.), whereas by far the highest concentration was observed in the control plants collected on the 3rd date (20.22 nmol·g^{-1} f.w.).

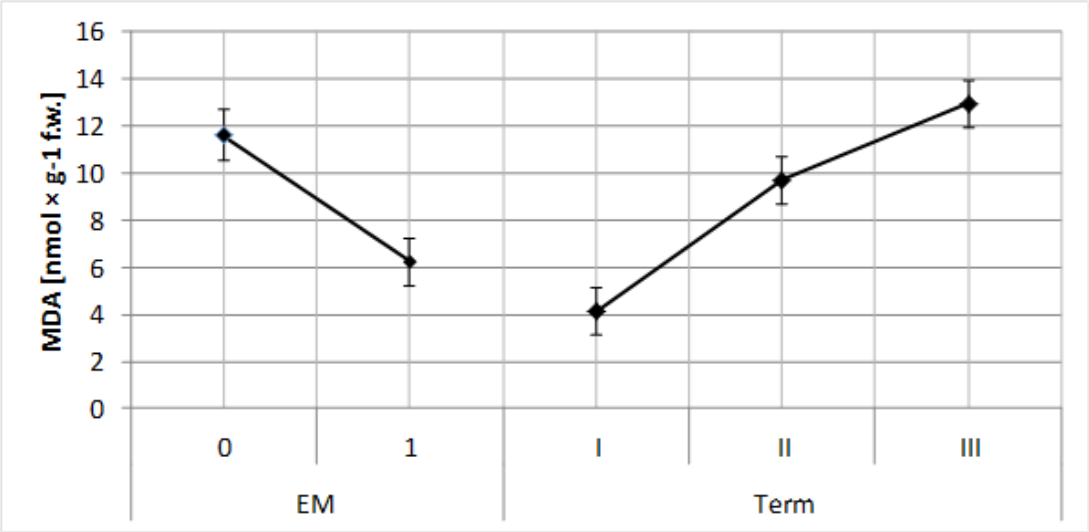

FIGURE 3
Average MDA concentration in sweet basil herb for individual experimental factors.

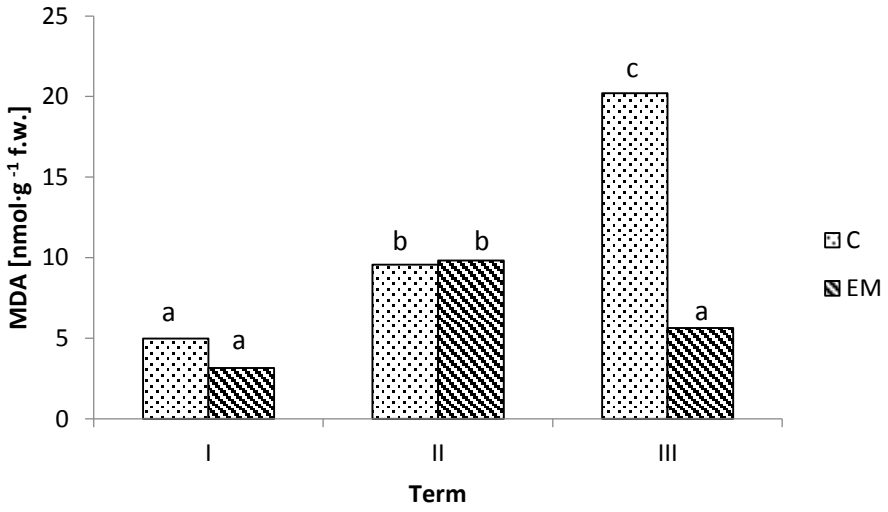

FIGURE 4
MDA content [nmol·g^{-1} f.w.] in the green parts of EM-treated sweet basil and under control conditions (without EM) depending in the time.

When comparing the MDA content in control plant herb and after EM application on respective dates of taking measurements, it was observed that the lowest concentration of this chemical compound was characteristic of the sweet basil plants being collected on the 1st date. However, the plants without EM contained significantly more MDA (5.01 $nmol \cdot g^{-1}$ f.w.), while those with EM less (3.17 $nmol \cdot g^{-1}$ f.w.). On the 2nd date, the MDA concentration increased and, regardless of the variant with EM, was at a similar level, i.e. 9.57 - 9.84 $nmol \cdot g^{-1}$ f.w. On the other hand, the MDA concentration in the control plants on the 3rd date was the highest (20.22 $nmol \cdot g^{-1}$ f.w.) and was significantly higher, 4-fold, than in the plants being treated with EM preparation (5.65 $nmol \cdot g^{-1}$ f.w.) – Fig. 4.

DISCUSSION

Due to the ongoing climate and habitat changes, the plants are exposed to the effects of abiotic factors that induce stress in them. To adapt to adverse conditions, the plants have developed some defence mechanisms that allow them to survive. Oxidative stress which is a response of the plant organism to the effects of stress-inducing stimulus is the phenomenon being most studied by scientists and best illustrates the condition of the test object. Bearing in mind the results presented in this paper, the significant increase of free proline content in sweet basil herb for the two variants of the material collected being collected on the third date should be taken into account in respect to other dates of making measurements. According to Koralewski [3], the proline level depends on both the internal environmental factors, such as plant age and its development stage, and the external ones, i.e. temperature, insolation, humidity, etc. Syversten and Smith [20] have demonstrated that the Pro content in the young plants is at the highest level than in the older ones – unlike in the present experiment. This may indicate the effects of a stress factor that activated the defence mechanism against free radicals. Since the function of proline includes, among others, osmoregulation, stabilisation of cell membranes and protection of plan tissues against degradation, the relevance of EM application in the analysed crop becomes essential. The obtained results show a significant reduction in the proline level in the plants being treated with EM preparation in relation to the control variant for the third date. Lower levels of this enzyme in the EM-treated plants may be justified by the presence of photosynthetic bacteria in the preparation which in co-operation with other micro-organisms provide plants with essential nutrients: amino acids, nucleic acids, bioactive substances and sugars [2]. The constant access to nutrients during stress allows protein degradation processes to slow down. In addition, EM are rich in micro-organisms that produce antioxidants, as well as in enzymes and hormones that support active cell division [1]. Talaat [2] in his experiment, has proven a mitigating effect of EM on the salt stress induced in common bean by increasing the protein synthesis and changing the composition of polyamines.

The effect of biologically active substances, i.e. bio-stimulators, on the proline content, other than that being observed in the present study, has been shown by Borowski and Blamowski [21]. They have observed a significant increase in the proline concentration in the leaves of *Ocimum basilicium* L. in the plants being treated with a bio-preparation compared to the control plants.

The MDA content in the analysed control variant is characterised by an upward trend over three months, which is consistent with the mechanism of organism aging. While on the first and the third date the MDA content in the EM-treated plants is significantly lower than in the control, the level of the analysed indicator on the second date seems to be striking. A similar level of this parameter for the two variants may indicate achieving optimum growing conditions and an adequate phenological phase by the plant in which the application of EM preparation does not bring significant changes. A significant reduction in the MDA level in the EM-treated plants in relation to the control on the third date points to the effects of antioxidants which, as reported by Higa [1], are the major product of EM.

A reduction in the values of oxidative stress parameters under optimum conditions as a result of the application of effective micro-organisms may be a confirmation of the protective properties of this preparation. According to Janas and Grzesik [22,23] biological conditioning of the seeds of some species of medicinal plants and vegetable crops enhances the health of seeds and improves their sowing value. On the other hand, Xu [24] and Chaudhry [25] have demonstrated the positive effects of the application of effective micro-organisms in maize growing. The bio-preparation has stimulated the growth of plants and induced their resistance and the process of photosynthesis [26].

CONCLUSION

The results obtained in this experiment confirm numerous scientific reports about the positive effect of EM on the growth and development of plants, not only under stress conditions. The study has shown a favourable effect of EM on the oxidative stress parameters in sweet basil by lowering the concentration of free proline and significant slowing down the process of lipid peroxidation in the plant tissues. Effective micro-organisms can be used in crop growing as a preparation to facilitate the adaptation of plants to changing climatic and habitat conditions.

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The influence of Effective Microorganisms and number of buds per cane in viticulture on chemical composition in fruits

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Summary

As a result of climate warming, wine-growing zones have moved to the north, where conditions exist may result in poor fruit quality. Fruits may develop significant amounts of tannin compounds, which are not acceptable to all consumers. The aim of this study was to demonstrate the influence of selected factors on the quality and content of polyphenols in grapevine fruits. The differentiating factors were as follows: two grapevine cultivars, varied number of buds per cane, and treatment with Effective Microorganisms (EM). To determine the total content of polyphenols and individual polyphenolic compounds in the tested fruits, the UPLC-PDA-MS method was used. The results indicated that the studied factors had no effect on total soluble solids and titratable acidity in grapes. The experiment revealed that polyphenol content was most dependent on the cultivar, followed by the number of buds per cane; EM treatment had the least effect. The fruit of the 'Regent' cultivar was characterised by higher polyphenol content. 'Cabernet Cortis' berries had higher levels of phenolic acids and flavan-3-ols, while 'Regent' berries were higher in anthocyanins and flavonols. EM treatment had a large impact on the reduction of tannic acid compounds. Fruits from untreated plants with four buds per cane had a significantly increased content of polyphenols, including flavan-3-ols.

Key words: polyphenol content, grapevine, Effective Microorganisms, buds per cane, tannin

Introduction

Extensive chemisation of agricultural fields has resulted in progressive environmental pollution, and the agents applied have exerted negative effects on the human body. Therefore, natural cultivation methods and preparations are being sought to ensure both bountiful and high-quality harvests (Javaid and Bajwa, 2011). Effective Microorganisms (EM) are biological preparations that contain selected, naturally occurring microbes, such as *Lactobacillus casei*, *Rhodopseudomonas palustris*, *Saccharomyces albus*, *Streptomyces albus*, and *Aspergillus oryzae* (Higa, 1989; Hu and Qi, 2013). The microorganisms in the preparations are capable of high levels of antioxidant production, and as such, they naturally aid the plant protection system. The EM technology was developed by Teruo Higa in the 1980s. The concept of using microbiological preparations in agriculture and environmental protection is thought to be an environmentally friendly alternative to commonly applied chemical agents (Higa, 1989).

Grapevine pruning and shrub training with a specific number of buds is the basic ampelotechnical treatment aimed at yielding high-quality fruits in vineyards (Brighenti et al., 2017). Pruning creates favourable conditions for the setting of properly sized fruits that are the right colour and have appropriate solid content. In addition, the procedure prevents damage that can be caused by shrubs becoming overloaded with too many fruits (Senthilkumar and Vijayakumar, 2015).

Polyphenols are one of the most frequently studied groups of biologically active compounds due to their health-promoting properties. At a time when cancer and cardiovascular disease are increasingly on the rise, polyphenols are a natural adjuvant in the treatment, as well as prevention, of such diseases. The biological activity and medicinal properties of polyphenols are associated with the diversity of their structures. The compounds classified as polyphenols demonstrate high antioxidant, anti-inflammatory, antibiotic, and anticarcinogenic activity (Mijowska et al., 2016).

The most frequently mentioned sources of this valuable group of compounds include red grapes and their processed products, primarily wines (Mijowska et al., 2017a). According to the International Organisation of Vine and Wine's 2017 statistical report on world vitiviniculture, the harvested output is approximately 77 million tons a year worldwide, of which 36% is consumed as fresh fruit and 47% is dedicated to wine production.

The polyphenol content in individual parts of the grapevine fruit is variable; the highest value is in the seeds at 60%-70%, with 28%-35% in the skin and approximately 10% in the fruit pulp (Godjevac et al., 2010). The structures of phenolic compounds in grapes have a significant effect on the formation of flavour and the aromatic properties of wines, as well as their colour, whereas anthocyanins play the most important role in the colour of grapes and wines (He and Giusti, 2010). The composition of the polyphenolic profile in grapes depends on many factors, such as the vine cultivar, degree of fruit ripeness, climatic and soil conditions, and physiological state of the plant. Interactions among the aforementioned agents make it difficult to isolate a specific effect of one agent on polyphenol content. Therefore, scientists and winemakers are unceasingly conducting research to investigate these factors (Pantelić et al., 2016).

From the consumer's point of view, a higher content of polyphenols is beneficial for health reasons. However, due to the wines taste properties, it would be advisable to obtain fruit with a reduced flavan-3-ol content. The aim of this experiment was to examine the effect of EM and the number of buds per cane on the quality of fruit grown in north-western Poland, including the content and profile of polyphenols. The study also investigated the relationships between the studied factors, which of the cultivation methods most effectively reduced the content of tannic compounds.

Material and methods

Location

The analysed material came from a non-irrigated five-year-old vineyard at the Research Station of West Pomeranian University of Technology in Szczecin. The site is in the north-western part of Poland called the Szczecin Lowland, approximately 90 km from the Baltic Sea. In this area, there are numerous hills the remnants of

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the frontal moraine that are 20-60 m above sea level. These hills affect the distribution and intensity of rainfall, number of sunlight hours, temperature, and wind speed. The climate is also significantly affected by the presence of large water basins (Szczecin Lagoon, Dąbie Lake, and the Odra River), which provide additional moisture during the vegetation period. The majority of the West Pomeranian Province belongs to zone 7A on Heinz and Schreiber's 'Map of zones of plant resistance to frost'. However, in Szczecin and in the nearby northern region, minimum temperatures range from -12 °C to -15 °C, which correspond to values typical of zone 7B (Mijowska et al., 2016).

Tab. 1 indicates changes in the weather for the years 2014 and 2015, as well as significant deviations from the average growing season during the multi-year period from 1951 to 2012. During the 2014 and 2015 growing seasons, average temperatures and sun hours were similar. Unusually low precipitation was observed during the 2015 growing season (242 mm), compared to the years 1951-2012 and 2014 (391 and 448 mm, respectively). The period from April to July 2015 was characterised by lower average temperatures and less sun hours relative to the same period in 2014. The largest weather deviation in 2015 took place in August, when rainfall was extremely low (14.7 mm) compared to the average rainfall for the growing season during the multi-year period and in 2014 (74.2 and 104.6 mm, respectively) (Mijowska et al., 2016).

The orchard contained an agricultural soil with a natural profile, developed from silt loam with an unusually low density of 1.12 Mg m^{-3}, a pH of 6.7, and a high water capacity of 31.3% (w w^{-1}). It also contained a high level of organic matter (29.2 g kg^{-1} soil). Regardless of the site, the soils were characterised by similar low salinity (EC 0.37 mS cm^{-1}). In comparison to optimal soil mineral content as determined by Sadowski et al. (1990), the soil in which the plants were grown, regardless of the stand, was characterised by high levels of phosphorus at 84 mg kg^{-1} (optimum 20-40 mg kg^{-1}), potassium at 93 mg kg^{-1} (optimum 50-80 mg kg^{-1}), and magnesium at 44 mg kg^{-1} (optimum 25-40 mg kg^{-1}). Every spring, nitrogen fertilisation was applied at a dose of 60 kg N per hectare, and a calcium fertiliser was applied at a dose of 80 kg Ca per hectare.

Grape samples

Two red grapevine cultivars, 'Regent' and 'Cabernet Cortis', were included in this research. The vines were planted with a north-south row orientation at 1 × 2.6 m and pruned with a Guyot (one-cane) training system. During cultivation, plants were treated with EM1, a beneficial microorganisms and molasses solution prepared according to the manufacturer's instructions. EM1 stock solution was diluted 1:1,000 (EM:water) and applied in spray form every second week, starting at the appearance of leaf buds and continuing until harvest. Additionally, the cultivars were grown with either four or eight buds per cane. The berry samples were collected in 2014 and 2015 at technological maturity.

Sampling was performed by picking grape berries randomly distributed throughout both cultivars. Each sample consisted of three replications each of 100 randomly selected berries on both sides of the canopy and from different parts of the clusters. The collected samples were immediately frozen at -25 °C until analysis.

Physicochemical parameters

Total soluble solid (TSS) content of grapes was determined as degrees Brix (°Bx) using a PAL-1 (Atago, Tokyo, Japan) refractometer. Titratable acidity (TA) was determined by titration of a water extract of juice with 0.1 N NaOH to an end point of pH 8.1.

Extraction procedure

Grape berries were prepared according to the method described in Oszmiański et al. (2013). The thawed berries were separated with methanol acidified with 2.0% formic acid. The separation was conducted two times by incubating the samples for 20 min under sonication (Sonic-6D, Polsonic, Warsaw, Poland), followed by shaking every 4 min. Subsequently, the suspension was centrifuged at 19,000 g for 10 min. Prior to the analysis, the supernatant was additionally purified with a 0.20 μm hydrophilic PTFE membrane (Millex Samplicity Filter, Merck).

The polyphenol content in each extract was determined by means of the ultraperformance liquid chromatography-photodiode array detector-mass spectrometry (UPLC-PDA-MS) method. All separations were conducted three times.

Identification of phenolic compounds by the UPLC-PDA-MS method

Analyses were performed according to the method described by Mijowska et al. (2016). In fruit extracts for both cultivars, polyphenols were identified using an ACQUITY UPLC System appointed with a binary solvent manager, a PDA detector (Waters Corporation, Milford, MA, USA) and a G2 Q-T of micro mass spectrometer (Waters Corporation, Manchester, UK) equipped with an electrospray ionisation source operating in the negative and positive modes. Individual polyphenols were separated using a UPLC BEH C18

Tab. 1: Weather conditions during the vegetative season (April-October) in the years 2014-2015 with reference to the average growing season during the multi-year period 1951-2012.

	month							
	IV	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	
Year	Average temperature (°C)							**Mean**
2014	10.8	13.4	16.3	21.3	17.5	15.4	11.8	15.2
2015	8.7	12.5	15.6	18.6	21.1	14.1	13.7	14.9
1951-2012	8.0	13.0	16.4	18.2	17.6	13.8	9.2	13.7
	Rainfall (mm)							**Total**
2014	47.5	85.3	26.5	70.8	104.6	80.9	32.8	448
2015	29.0	48.0	32.8	62.0	14.7	34.4	22.1	242
1951-2012	39.7	62.9	48.2	69.6	74.2	58.7	37.3	391
	Sun hours							**Total**
2014	210	213	189	224	123	133	99	1191
2015	136	161	159	197	278	126	131	1188

column (1.7 µm, 2.1 mm × 100 mm, Waters Corporation, Milford, MA, USA). Based on the data obtained, software was developed to scan multiple samples for defined substances. The various data analyses were monitored at the following wavelengths: flavan-3-ols at 280 nm, phenolic acids at 320 nm, flavonol glycosides at 360 nm, and anthocyanins at 520 nm. The PDA spectra were measured over a wavelength range of 200 - 600 nm in 2 nm increments. Finally, the retention times and spectra were compared with authenticated standards.

Statistical analysis

Analysis of variance (ANOVA) was carried out in order to estimate the influence of three different factors (cultivar, number of buds, and EM) on the physicochemical attributes of grapes. This method also allowed the statistical significance of the physical effect of particular factors, measured as contribution percentage, to be calculated (Davim and Reis, 2003). Mean comparisons were performed using Tukey's least significant difference (LSD) test with significance set at $p < 0.05$. The statistical analyses were performed using the Statistica 12.5 software.

Results and discussion

Total soluble solids (TSS) and titratable acidity (TA)

Statistical analysis demonstrated that the performed treatments (i.e., the number of buds per cane and EM treatment) had no significant effect on TSS (Tab. 2). The average TSS content in the fruits of both cultivars ranged from 16.85°Bx to 17.95°Bx, with the TA ranging from 0.525 to 0.640 mg 100 g^{-1} (Tab. 3).

TSS and TA are very important parameters in evaluating the usefulness of the fruit in processing. In Poland's climatic conditions, fruits of the cultivated vine cultivars contain 18% sugar on average, representing approximately 22% of TSS (Angelov et al., 2015).

Leão et al. (2016) concluded that the cultivar selected determines the method of shrub training (i.e., the number of buds per cane). Gladstones (1992) demonstrated that fruits that develop on vines with loose crowns feature a higher concentration of sugar in the juice and a better acid balance, unlike fruits that develop in shaded crown conditions. The results obtained in this experiment indicated that increased crown density had no significant effect on TSS and TA.

Polyphenols

The identification of 36 compounds categorised as anthocyanins, phenolic acids, flavanols, and flavan-3-ols was based on a comparison of their retention times, MS data, and MS/MS data with available standards and published data (Tab. 5-6). The research indicated that the studied factors had a significant effect on the total content of polyphenols in fruits (Tab. 4). The most significant factor that differentiated polyphenol content was the cultivar (P = 69.8%), followed by the number of buds (P = 10.8%) and the use of EM (P = 7.9%). Significant interaction was demonstrated between the ampelotechnical treatments applied for both vine cultivars. A significantly lower polyphenol content was found in fruits from plants treated with EM and trained with eight buds per cane 382.4 mg 100 g^{-1} of fresh weight (FW) for 'Regent' and 317.29 mg 100 g^{-1} FW for 'Cabernet Cortis', as compared to those from plants in the control conditions and rooted with four buds per cane (528.73 mg 100 g^{-1} FW for 'Regent' and 382.42 mg 100 g^{-1} FW for 'Cabernet Cortis') (Tab. 5). For vines rooted with four buds, as compared to vines with eight buds, the average total polyphenol content was higher by approximately 10.4% for 'Regent' and 11.4% for 'Cabernet Cortis'. The error value (P = 10.2%) indicates the magnitude of the effect exerted by other untested external factors on the measured parameters (Tab. 4).

The 'Regent' cultivar features a significantly higher total content of polyphenols in fruits (472.01 mg 100 g^{-1} FW), as compared to the 'Cabernet Cortis' cultivar (349.83 mg 100 g^{-1} FW), regardless of the treatments performed (Fig. 1). These values were more than twice as high as those of the fruits of 'Cabernet Sauvignon' (2356 mg kg^{-1}) and 'Tempranillo' (1489 mg kg^{-1}) cultivated in southern Europe (Guerrero et al., 2009). The quantity of polyphenols and

Tab. 2: ANOVA table for total soluble solids and titratable acidity.

	Total soluble solids		Titratable acidity	
Source of variance	p	P (%)	p	P (%)
a	0.943	0.06	0.581	3.86
b	0.966	0.02	0.937	0.08
c	0.720	1.56	0.937	0.08
a × b	0.579	3.79	0.987	0.00
a × c	0.943	0.06	0.861	0.38
b × c	0.874	0.30	0.715	1.67
a × b × c	0.579	3.79	0.836	0.53
Error		90.42		93.40
Total		100.00		100.00

a – cultivar; b – number of buds per cane; c – EM treatment; p – probability of error, P (%) – percentage of contribution

Tab. 3: Effect of the performed treatments on the total soluble solids (in °Bx) and titratable acidity (in g L^{-1}) in the fruit of the tested vine cultivars. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level. Lowercase letters (a) indicate group means; capital letters (A) indicate group averages.

	'Regent'		average	'Cabernet Cortis'		average
	C	EM		C	EM	
	Total soluble solids (°Bx)					
4	16.45 a	17.65 a	**17.05 A**	18.15 a	17.65 a	**17.90 A**
8	18.05 a	17.85 a	**17.95 A**	15.85 a	17.85 a	**16.85 A**
average	**17.25 A**	**17.75 A**		**17.00 A**	**17.75 A**	
	Titratable acidity (g L^{-1})					
4	0.595 a	0.660 a	**0.627 A**	0.580 a	0.585 a	**0.525 A**
8	0.645 a	0.620 a	**0.632 A**	0.600 a	0.580 a	**0.590 A**
average	**0.620 A**	**0.640 A**		**0.590 A**	**0.583 A**	

C – control treatment; EM – treatment with EM; 4,8 – number of buds per cane

Tab. 4: ANOVA table for polyphenol content.

	Polyphenols		**Anthocyanins**		**Phenolic acids**		**Flavonols**		**Flavan-3-ols**	
Source of variance	p	P (%)	p	P (%)	p	P(%)	p	P(%)	p	P(%)
a	0.000	69.82	0.000	87.87	0.000	28.46	0.000	52.79	0.000	28.56
b	0.000	10.79	0.000	3.09	0.394	0.55	0.036	1.89	0.000	41.42
c	0.000	7.85	0.001	2.65	0.002	8.79	0.000	26.22	0.001	8.56
a × b	0.474	0.22	0.116	0.48	0.000	43.20	0.636	0.09	0.110	1.55
a × c	0.122	1.09	0.009	1.47	0.739	0.08	0.000	9.57	0.034	2.85
b × c	0.875	0.01	0.555	0.06	0.295	0.83	0.410	0.27	0.023	3.32
a × b × c	0.898	0.01	0.579	0.06	0.337	0.69	0.849	0.01	0.544	0.21
Error		10.20		4.31		17.39		9.16		13.53
Total		100.00		100.00		100.00		100.00		100.00

a – cultivar; b – number of buds per cane; c – EM treatment; p – probability of error; P (%) – percentage of contribution

Tab. 5: Effect of the performed treatments on polyphenol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

	'Regent'		**average**	'Cabernet Cortis'		**average**
	C	EM		C	EM	
	Polyphenols (mg 100 g^{-1} FW)					
4	528.73 a	469.98 ab	**499.35 A**	382.42 cd	357.01 cde	**369.71 C**
8	471.28 ab	418.03 bc	**444.66 B**	342.57 de	317.29 e	**329.93 D**
average	**500.01 A**	**444.00 B**		**362.50 C**	**337.15 C**	
	Anthocyanins (mg 100 g^{-1} FW)					
4	374.28 a	333.06 bc	**353.67 A**	206.38 d	207.50 d	**206.94 C**
8	338.81 ab	297.14 c	**317.98 B**	198.01 d	184.77 d	**191.39 C**
average	**356.55 A**	**315.10 B**		**202.20 C**	**196.14 C**	
	Phenolic acids (mg 100 g^{-1} FW)					
4	23.13 a	22.18 a	**22.66 A**	36.37 c	33.14 cd	**34.76 C**
8	30.83 bcd	26.34 ab	**28.59 B**	29.02 bc	25.64 ab	**27.33 B**
average	**26.98 AB**	**24.26 A**		**32.69 C**	**29.39 BC**	
	Flavonols (mg 100 g^{-1} FW)					
4	24.33 a	15.62 b	**19.98 A**	13.73 bc	11.34 c	**12.54 B**
8	22.06 a	14.60 bc	**18.33 A**	12.28 bc	10.67 c	**11.47 B**
average	**23.20 A**	**15.11 B**		**13.00 BC**	**11.01 C**	
	Flavan-3-ole (mg 100 g^{-1} FW)					
4	106.99 a	99.11 a	**103.05 A**	125.94 c	105.02 a	**115.48 C**
8	79.58 b	79.94 b	**79.76 B**	103.27 a	96.21 a	**99.74 A**
average	**93.28 AB**	**89.53 A**		**114.60 C**	**100.62 B**	

their composition in fruits depends on the cumulative parts (i.e., peel, flesh, and seeds). This is characteristic for a given cultivar (Pantelić et al., 2016).

As indicated in this experiment, the better lighting conditions of vines rooted with a smaller number of buds resulted in a higher polyphenol content in the fruits. This corresponds to the findings of other authors (Degu et al., 2016; Haselgrove et al., 2000).

Due to the process-related usefulness of the yield, the content of polyphenol compounds is one of the most important parameters of quality in grapes and wines. Polyphenols have a direct impact on the organoleptic characteristics of wines, such as taste, sourness, bitterness, and colour (Garrido and Borges, 2013). They are also strong antioxidants, which are valuable to human health. Therefore, the results obtained after treatment with EM contradict the expectations of both producers and consumers. The use of EM in cultivation caused a significant decrease in total polyphenol content in grapes, regardless of the selected cultivar and type of pruning. From a physiological point of view, this reduction creates favourable conditions for plants. EM is a natural protective preparation designed to relieve physiological plant stress caused by various external factors (Higa, 1989; Talaat, 2014). However, increased levels of antioxidants in plants often indicate initiation of defence processes as a response to the intensified action of free radicals (Talaat et al., 2015).

Anthocyanins

Anthocyanins were the largest group of polyphenolic compounds analysed in the fruits of both cultivars (Tab. 6-7). Anthocyanins, classified as a flavonoid subgroup, are natural plant pigments that may appear mainly as violet, dark blue, and red. Anthocyanin accumulation in grape skins was significantly higher at 20 °C than

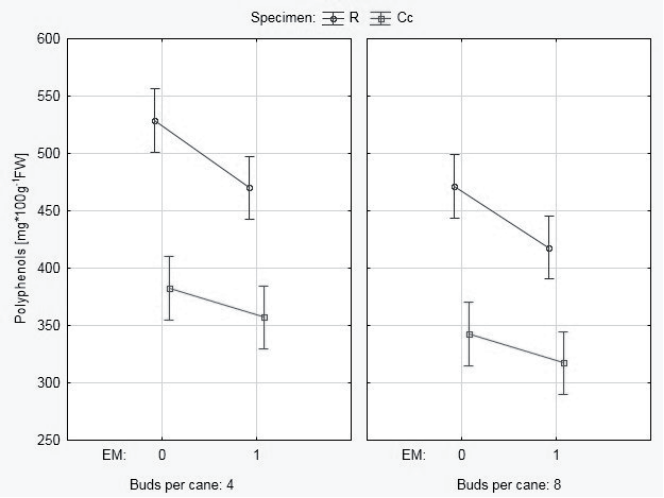

Fig. 1: Effect of the performed treatments on total polyphenol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

at 30 °C, and the most temperature-sensitive stage was from one to three weeks after colouring began (YAMANE et al., 2006).

The analysis of variance showed that the total content of anthocyanins was significantly affected by the cultivar, the number of buds per cane, and EM treatment, as well as the interaction between the number of buds and EM treatment (Tab. 4). The cultivar was found to have the highest percentage share (87.9%) of influence. The shares for the other factors (i.e., EM and the number of buds) were 2.65% and 3.1%, respectively. As in the case of total polyphenols, the error value (P = 4.3%) was higher than the P values for EM treatment and the number of buds; this indicates that untested factors had a greater influence on anthocyanin content.

Apart from its bioactive properties, anthocyanins are particularly important in the technology of red wine production (MIJOWSKA et al., 2017a). For the tested grapes, 19 groups of these compounds were identified. In fruits of the 'Regent' cultivar, the compounds amounted to more than 70% and, for 'Cabernet Cortis', 53.8% to 58.2% of all polyphenolic compounds (Tab. 6-7). The most abundant anthocyanins in the 'Regent' cultivar fruits were the 3-*O*-glucoside forms of petunidin, peonidin, delphinidin, malvidin, and cyanidin. These compounds are among the most important anthocyanins in grapes (IVANOVA et al., 2010). According to other authors, malvidin derivatives are the largest group of anthocyanin compounds in red grape cultivars (FIGUEIREDO-GONZÁLEZ et al., 2012). The total anthocyanin content in the 'Regent' cultivar fruits was 335.83 mg 100 g^{-1} FW and, for 'Cabernet Cortis', 199.17 mg 100 g^{-1} FW (Fig. 2). They represented 71.2% and 56.9%, respectively, of the polyphenol content in fruits of the tested vine cultivars. For the 'Regent' cultivar, the highest quantity of anthocyanins (374.28 mg 100 g^{-1} FW) was found in the fruits of vines rooted with four buds per cane and in controlled conditions. A significant decrease was observed after EM treatment: from 338.81 to 297.14 mg 100 g^{-1} FW with eight buds and from 374.27 to 333.06 mg 100 g^{-1} FW with four buds (Tab. 6). For 'Cabernet Cortis', the performed treatments produced no significant changes in anthocyanin content (Tab. 6).

Environmental factors have a greater influence on the levels of anthocyanins than on their composition, which is more closely related to the vine cultivar. Anthocyanins are the main cause of the red colour of fruits, and they must be extracted from such fruits. They are accumulated primarily in the fruit skin and, in some cultivars, also in the flesh (FLAMINI et al., 2013). This study confirms that anthocyanin composition is characteristic of the cultivar, as determined by the research conducted by other authors.

Shrubs with different numbers of buds feature different crown microclimates. The type of pruning applied results in changes in the levels of solar radiation, temperature, humidity, and wind (SMART, 1987), affecting the composition and quality of grapes. In the completed experiment, thinning out the vine crown had a positive effect on the content of anthocyanins in fruits. Similar results were obtained by ASELGROVE et al. (2000) and MIJOWSKA et al. (2016).

According to TALAAT (2014), EM preparations support the detoxification mechanism in plants exposed to stress. In plants subjected to EM, an increased ability to sweep H_2O_2 in the glutathione-ascorbate cycle was observed. This can effectively attenuate the plants' defensive reaction to stress. In this research, the Cabernet Cortis cultivar exhibited similar behaviour after EM treatment. However, from the producer's and consumer's viewpoints, a lower anthocyanin content in fruits is undesirable.

Phenolic acids

The profile of phenolic acids in the fruits of the studied cultivars was diversified and depended on the agrotechnical treatments performed. Phenolic acids represented 5.4% of the total polyphenol content for the 'Regent' cultivar and 8.9% for the 'Cabernet Cortis' cultivar. The cultivar, EM treatment, and the interaction between the cultivar and the number of buds per cane had a significant effect on the content of phenolic acids (Tab. 4). The greatest influence was exerted by the interaction between the cultivar and the number of buds (P = 43.2%), followed by the cultivar (P = 28.5%) and treatment with EM (P = 8.8%).

Pruning the 'Regent' cultivar with eight buds in controlled conditions increased the phenolic acid content in the fruits. In turn, pruning the 'Cabernet Cortis' cultivar with a higher number of buds per cane and treating with EM resulted in a significant decrease in the content of phenolic acids (Tab. 5-6). The average concentration of phenolic acids in both vine cultivars was determined by the number of buds per cane, not by treatment with EM. For the 'Regent' cultivar with eight buds, significantly higher levels of phenolic acids were found, while a comparable result was obtained for 'Cabernet Cortis' vines with four buds per cane (Fig. 3). Similar relationships were observed by MIJOWSKA et al. (2016), who demonstrated that less shading of the shrub crown significantly decreased the phenolic acid content in the 'Regent' cultivar. EHRHARDT et al. (2014) found that the level of trans-caftaric acid depended on the cultivation site, with 12.70 mg kg^{-1} FW in 'Regent' grapes harvested in Italy and a much higher value (35.98 mg kg^{-1} FW) in the colder climate of Germany. In the authors' own research, the results for the 'Regent' fruits ranged from 18.84 mg 100 g^{-1} FW (four buds plus EM treatment) to 26.32 mg 100 g^{-1} FW (eight buds).

Flavonols

Flavonols are yellow pigments masked by anthocyanins in red wines; however, the pigments affect their colour by co-pigmentation (CASTILLO-MUÑOZ et al., 2010). As a result, the extraction of anthocyanins during wine production is enhanced (CASTILLO-MUÑOZ et al., 2007). The greatest biosynthesis of flavonols is observed during the intensive ripening of fruits that occurs post-veraison (MATTIVI et al., 2006).

In the fruits of the 'Regent' and 'Cabernet Cortis' cultivars, flavonols represented 4.1% and 3.4%, respectively, of the total polyphenols detected. The analysis of variance revealed that the performed treatments had a significant impact on the flavonol content in fruits. An interaction was observed between the cultivar and EM treatment (Tab. 4-5). Once again, the most crucial factor in determining the content of flavonols was the vine cultivar (P = 52.8%), followed by treatment with EM (P = 26.2%) and the interaction (P = 9.6%). The number of buds per cane (P = 1.9%) had a small but significant

Tab. 6: Polyphenol content (in mg 100 g^{-1} FW) of grape cultivar 'Regent'. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

	R8	R4	R8 EM	R4 EM
Compounds (mg 100 g^{-1})				
Delphinidin 3.5-diGlc	5.39 ab	6.33 b	4.79 a	5.67 ab
Delphinidin 3-O-Glc;Petunidin 3.5-diGlc	147.08 ab	161.01 b	127.64 a	142.45 ab
Peonidin 3.5-diGlc;Malvidin 3.5-diGlc;Cyanidin 3-O-Glc	112.78 ab	136.19 c	97.93 a	117.23 bc
Petunidin 3-O-Glc	21.27 b	20.78 b	13.68 a	11.09 a
Peonidin 3-O-Glc	16.22 b	13.58 a	12.88 a	13.75 a
Malvidin 3-O-Glc	22.25 a	20.53 a	27.94 b	28.74 b
Delphinidin 3-O-acetyl-Glc	0.90 a	1.39 b	0.79 a	1.23 b
Delphinidin 3-O-caffeoyl-Glc	0.71 ab	0.95 c	0.62 a	0.84 bc
Petunidin 3-O-acetyl-Glc	0.51 ab	0.64 c	0.45 a	0.56 bc
Cyanidin 3-O-caffeoyl-Glc	0.11 ab	0.13 b	0.10 a	0.11 ab
Petunidin 3-O-caffeoyl-Glc	0.44 ab	0.57 c	0.39 a	0.51 bc
Malvidin 3-O-acetyl-Glc	0.24 ab	0.30 c	0.21 a	0.27 bc
Delphinidin 3-O-coumaroyl-Glc	3.28 b	3.68 c	2.89 a	3.25 b
Peonidin 3-O-coumaroyl-Glc	0.82 c	0.70 b	0.72 b	0.62 a
Peonidin 3-O-caffeoyl-Glc	0.11 a	0.34 b	0.10 a	0.30 b
Malvidin 3-O-caffeoyl-Glc	1.53 ab	1.82 c	1.35 a	1.61 bc
Cyanidin 3-O-coumaroyl-Glc	1.64 bc	1.72 c	1.44 a	1.52 ab
Petunidin 3-O-coumaroyl-Glc	0.07 ab	0.08 b	0.06 a	0.07 ab
Malvidin 3-O-coumaroyl-Glc	3.47 a	3.52 a	3.19 a	3.23 a
Anthocyanins sum	**338.81 b**	**374.27 c**	**297.14 a**	**333.06 b**
GRP (cis- and trans-isomers	3.32 c	2.77 ab	2.92 bc	2.45 a
Caftaric acid (cis- and trans-isomers)	26.32 c	19.35 a	22.37 b	18.84 a
Coutaric acid (cis- and trans-isomers	0.30 b	0.22 a	0.27 b	0.20 a
Fertaric acid	0.07 a	0.11 b	0.06 a	0.10 b
Galic acid	0.82 c	0.68 ab	0.72 b	0.60 a
Phenolic acids sum	**30.83 c**	**23.13 ab**	**26.34 bc**	**22.18 a**
Myricetin glucoside	4.50 b	5.18 c	3.95 a	4.58 b
Myricetin glucuronidec	0.82 b	0.75 b	0.39 a	0.45 a
Rutin	1.66 a	1.63 a	1.46 a	1.44 a
Quercetin-3-Oglucoside	1.10 ab	1.29 b	0.97 a	1.14 ab
Quercetin glucuronide	13.90 b	15.38 b	7.76 a	7.92 a
Quercetin	0.09 ab	0.11 b	0.08 a	0.09 ab
Flavonols sum	**22.06 b**	**24.33 b**	**14.60 a**	**15.62 a**
Dimer B1	1.10 b	0.66 a	2.08 c	0.58 a
Catechin	3.56 c	2.74 ab	3.13 bc	2.42 a
Dimer B2	11.26 ab	12.43 b	9.90 a	11.48 ab
Epicatechin	51.06 a	78.58 b	53.51 a	73.23 b
Galloylated dimer	4.51 ab	5.12 b	3.97 a	4.52 ab
Dimer B4	8.08 b	7.46 ab	7.35 ab	6.88 a
Flavan-3-ols sum	**79.58 a**	**106.99 c**	**79.94 a**	**99.11 b**
TOTAL	**471.29 B**	**528.73 C**	**418.03 A**	**469.98 B**

C – control treatment; EM – treatment with EM; 4,8 – number of buds per cane

influence (Tab. 4). After EM treatment, a significant decrease in flavonol content was found in the 'Regent' cultivar (Tab. 5-7, Fig. 4). As in the research by MIJOWSKA et al. (2016), this study found that the crown structure (pruning type) did not affect the content of flavonols in that cultivar. In turn, neither treatment procedure had a significant influence on flavonol content for the 'Cabernet Cortis' cultivar (Tab. 5).

The flavonol group includes, in particular, quercetin and its derivatives, myricetin, kaempferol, and isorhamnetin. The quantity of flavonols largely depends on the vine cultivar (LIANG et al., 2011). The 'Regent' fruits were more abundant in quercetin derivatives, including rutin, as compared to the fruits of the 'Cabernet Cortis' cultivar. Taking into account the significant decrease in flavonol content in the 'Regent' fruits after EM treatment and the previously mentioned reports by TALAAT (2014) relating to the detoxifying properties of the preparation, a hypothesis can be constructed that quercetin derivatives have a significant effect on the glutathione-ascorbate cycle in the plant. Moreover, this may indicate a significant

Tab. 7: Polyphenol content (in mg 100 g^{-1} FW) of grape cultivar 'Cabernet Cortis'. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

	Cc8	Cc4	Cc8 EM	Cc4 EM
Compounds (mg 100 g^{-1})				
Delphinidin 3.5-diGlc	4.78 c	3.41 ab	3.65 b	2.97 a
Delphinidin 3-O-Glc;Petunidin 3.5-diGlc	81.43 ab	94.52 c	75.29 a	89.21 bc
Peonidin 3.5-diGlc;Malvidin 3.5-diGlc;Cyanidin 3-O-Glc	49.78 b	50.89 b	46.66 a	56.07 c
Petunidin 3-O-Glc	14.21 c	10.21 a	11.87 ab	12.73 ab
Peonidin 3-O-Glc	5.16 a	7.37 b	5.05 a	9.45 c
Malvidin 3-O-Glc	22.30 b	18.22 a	23.97 b	18.68 a
Delphinidin 3-O-acetyl-Glc	3.90 b	4.61 c	3.21 a	3.82 b
Delphinidin 3-O-caffeoyl-Glc	1.24 a	3.38 b	1.11 a	1.32 a
Petunidin 3-O-acetyl-Glc	1.29 a	1.03 a	1.26 a	1.00 a
Cyanidin 3-O-caffeoyl-Glc	2.27 b	1.96 a	2.02 a	1.96 a
Petunidin 3-O-caffeoyl-Glc	0.14 a	0.19 b	0.14 a	0.15 a
Malvidin 3-O-acetyl-Glc	0.26 ab	0.29 b	0.22 a	0.25 ab
Delphinidin 3-O-coumaroyl-Glc	5.62 c	4.91 ab	5.18 b	4.88 a
Peonidin 3-O-coumaroyl-Glc	0.56 a	0.70 b	0.55 a	0.56 a
Peonidin 3-O-caffeoyl-Glc	0.19 b	0.15 a	0.19 b	0.16 a
Malvidin 3-O-caffeoyl-Glc	0.68 b	0.64 b	0.51 a	0.61 b
Cyanidin 3-O-coumaroyl-Glc	1.15 a	1.07 a	1.12 a	1.02 a
Petunidin 3-O-coumaroyl-Glc	0.11 a	0.18 b	0.11 a	0.10 a
Malvidin 3-O-coumaroyl-Glc	2.92 b	2.67 a	2.66 a	2.58 a
Anthocyanins sum	**198.01 ab**	**206.38 b**	**184.77 a**	**207.50 b**
GRP (cis- and trans-isomers	1.95 b	1.74 a	1.92 b	1.71 a
Caftaric acid (cis- and trans-isomers)	26.19 ab	33.82 c	22.85 a	30.67 bc
Coutaric acid (cis- and trans-isomers	0.41 a	0.42 a	0.39 a	0.37 a
Fertaric acid	0.05 a	0.05 a	0.08 b	0.04 a
Galic acid	0.42 b	0.34 a	0.41 b	0.35 a
Phenolic acids sum	**29.02 b**	**36.37 c**	**25.64 a**	**33.14 bc**
Myricetin glucoside	4.35 a	5.15 b	5.29 b	3.89 a
Myricetin glucuronidec	0.91 b	0.82 ab	0.48 a	0.80 a
Rutin	0.36 a	0.51 c	0.45 bc	0.38 ab
Quercetin-3-Oglucoside	0.52 a	0.55 a	0.50 a	0.48 a
Quercetin glucuronide	6.04 cd	6.65 d	3.87 a	5.74 bc
Quercetin	0.10 b	0.05 a	0.09 b	0.05 a
Flavonols sum	**12.28 ab**	**13.73 b**	**10.67 a**	**11.34 ab**
Dimer B1	1.42 b	1.44 b	1.28 a	1.30 a
Catechin	2.05 b	1.99 ab	1.59 a	1.85 ab
Dimer B2	11.56 a	13.11 b	10.30 a	11.12 a
Epicatechin	76.70 b	98.59 c	67.60 a	76.28 b
Galloylated dimer	7.37 a	9.19 b	9.28 b	8.61 b
Dimer B4	4.18 b	1.61 a	6.16 c	5.86 c
Flavan-3-ols sum	**103.27 ab**	**125.94 c**	**96.21 a**	**105.02 b**
TOTAL	**342.57 B**	**383.76 C**	**317.29 A**	**357.92 B**

Cc – 'Cabernet Cortis'; 4,8 – numbers of buds per cane; EM – treatment with EM.

effect of EM on quercetin derivatives. This hypothesis could also justify the strong correlation between the cultivar and EM treatment. Varying the number of buds in the experiment did not have a significant influence on the content of flavonols. Different results were obtained by SPAYD et al. (2002), who showed that well insolated fruits of the 'Merlot' cultivar contained almost 10 times the total concentration of flavonols as those harvested from shaded clusters. In addition, BAIANO et al. (2015), FENG et al. (2015), and MIJOWSKA et al. (2016) presented the beneficial effect of thinning out the vine crown on flavonol content. Considering the error value of P = 6.4%, the lack of influence of the number of buds on flavonol content during the experiment may have been caused by other external factors excluded from the research.

Flavan-3-ols

Flavan-3-ols are a group of tannin compounds that plentifully occur mainly in red grapes. They are the cause of the 'structure' of wine,

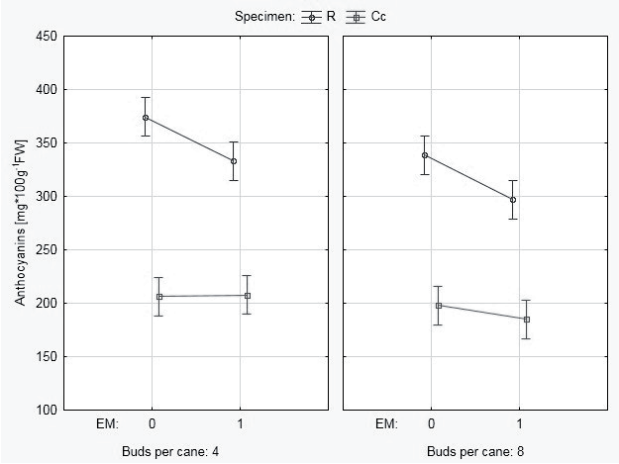

Fig. 2: Effect of the performed treatments on anthocyanin content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

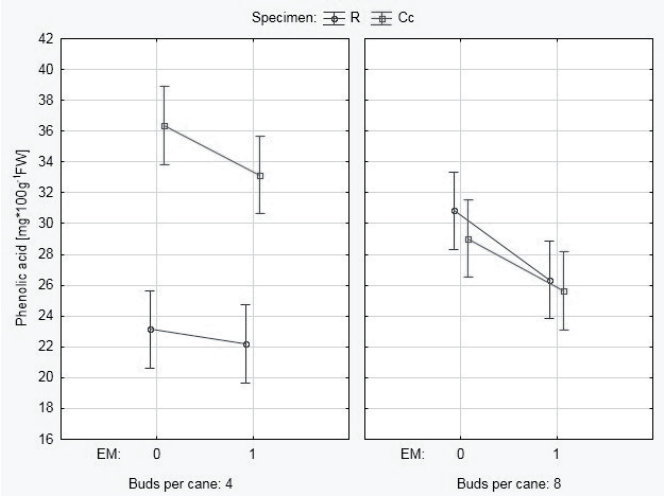

Fig. 3: Effect of the performed treatments on phenolic acid content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

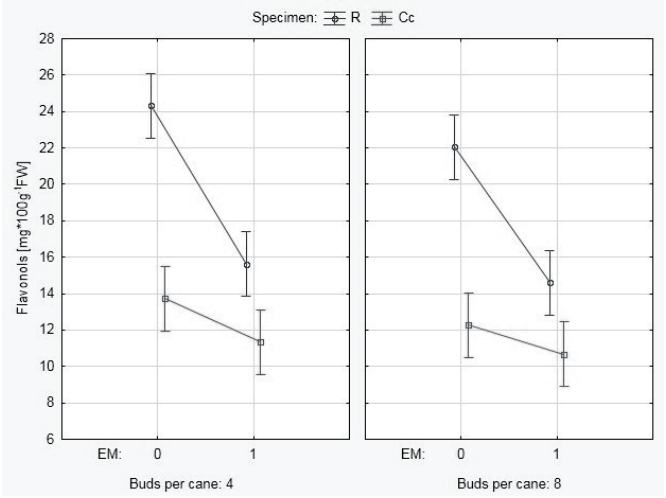

Fig. 4: Effect of the performed treatments on flavonol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

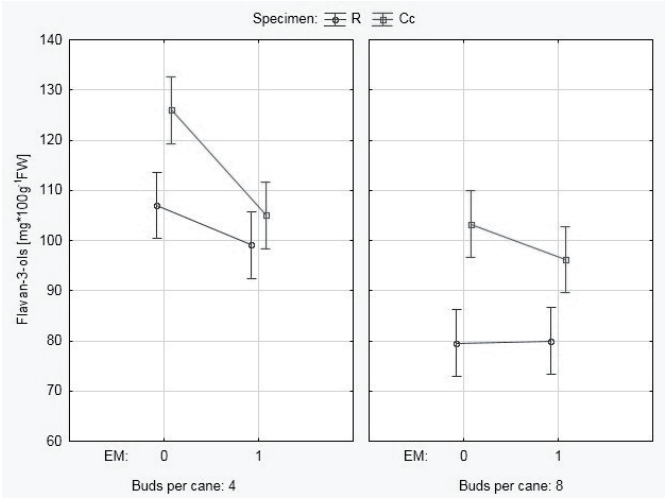

Fig. 5: Effect of the performed treatments on flavan-3-ol content (in mg 100 g^{-1} FW) in the fruits of the tested vine cultivars.

its astringency, and its bitterness. They also play a very important role in stabilising the red colour of wine (Guerrero et al., 2009). During the maceration process, the compounds undergo extraction and pass on to the must. In wine prepared from the 'Regent' cultivar, flavan-3-ol content was approximately 70.4 mg 100 g^{-1} (Mijowska et al., 2017b). This research found that the total content of flavan-3-ols in the 'Regent' fruits was 91.4 mg 100 g^{-1} FW, and 107.61 mg 100 g^{-1} FW for 'Cabernet Cortis'. Therefore, the compounds from this group represented 19.4% and 30.8%, respectively, of the total polyphenol content detected in the fruits of these vine cultivars. The analysis of variance demonstrated that total flavan-3-ol content was significantly influenced by the cultivar (P = 28.6%), the number of buds (P = 41.4%), EM treatment (P = 8.6%), and the interaction between pruning and EM treatment (P = 3.3%) as well as between the cultivar and EM treatment (P = 2.9%) (Tab. 4). In the fruits of both vine cultivars trained with a larger number of buds, the average flavan-3-ol content was significantly lower than the fruits of shrubs trained with fewer buds per cane (Fig. 5). EM treatment resulted in a significant decrease in flavan-3-ol content in the 'Cabernet Cortis' cultivar.

The content of flavan-3-ols in grapes depends on the cultivar; in the individual parts of the fruit, the content is highest in the seeds, followed in descending order by the skin, stick, and flesh (Pantelić et al., 2016). Reduced shrub crown density contributed to an increase in flavan-3-ol content in fruits, as reported by Mijowska et al. (2016) and Reynolds and Vanden Heuvel (2009). Furthermore, Degu et al. (2016) concluded that light had no effect on the content of flavan-3-ols. As reported by Li et al. (2008), flavan-3-ols are recognised as the most sensitive of all flavonoids to non-enzymatic degradation processes. During fermentation, such compounds (e.g., catechins) may undergo partial splitting into phenolic units with a lower molecular mass. This process is mainly due to a temperature rise (Garrido and Borges, 2013). A change in the quantity of these compounds was also observed during exposure to UVC radiation (Mijowska et al., 2017a). The compounds of this group most frequently found in grapes are epicatechins, with levels ranging from 51.06 to 78.58 mg 100 g^{-1} FW for the 'Regent' cultivar, depending on the agrotechnical treatments applied. For 'Cabernet Cortis' fruits, the content was higher (67.60 to 98.59 mg 100 g^{-1} FW) (Tab. 6-7). The high content of the compound in fruits was confirmed by the research completed by Ehrhardt et al. (2014) and Mijowska et al. (2017a).

Daly and Stewart (1999) concluded that EM treatment can improve

growth and yield by increasing photosynthesis and producing bioactive substances, such as hormones and enzymes. Therefore, the reduction in flavan-3-ol content in fruits by means of EM may be justified, once again, by the antioxidant properties of the preparation.

Conclusion

The experiment demonstrated that the number of buds per cane and the use of EM had a significant effect on polyphenol content in the fruits of the wine grape cultivars studied. However, these factors had no significant effect on TA and TSS. The polyphenol content in the fruits was determined mainly by the cultivar and, to a lesser extent, the number of buds per cane and the smallest use of EM. The fruits of the 'Regent' cultivar were characterised by a higher polyphenol content compared to those of the 'Cabernet Cortis' cultivar. 'Cabernet Cortis' berries had higher levels of phenolic acids and flavan-3-ols, while those of 'Regent' had higher levels of anthocyanins and flavonols. Pruning plants with four buds per cane increased the content of all polyphenol groups studied, with the exception of phenolic acids in the 'Regent' cultivar. The use of EM reduced polyphenols, especially tannin compounds, in the fruits of both grape cultivars. From the point of view of wine production in a cold climate, this phenomenon is desirable and beneficial. Keeping the vines at four buds per cane without using EM mostly increased the levels of polyphenols, including the flavan-3-ols. However, leaving eight buds per cane and using EM contributed to a significant reduction of these compounds, as well as to a lower concentration of flavan-3-ols.
Selecting the proper cultivar and pruning the vine shrubs with a smaller number of buds per cane is conducive to obtaining fruits of higher quality and polyphenol content.

Author's contribution:
AA experimental design, field work and data collection, laboratory work, data analysis, redaction of manuscript; IO supervision, experimental design, data analysis; JW supervision, data analysis; JO laboratory work, data collection.

Conflict of interest disclosure: The authors declare no conflict of interest related to this work.

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EFFECT OF TYTANIT® ON THE PHYSIOLOGICAL ACTIVITY OF WILD STRAWBERRY (*FRAGARIA VESCA* L.) GROWN IN SALINITY CONDITIONS

– Research paper –

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Abstract: Progressive global warming and decreasing freshwater resources are forcing to look for alternative solutions in plants cultivation. The use of saltwater and cultivation in saline areas becomes increasingly common. Wild strawberry is a rich source of antioxidant compounds beneficial for human health. The aim of the study was to investigate the effect of Tytanit® on the physiological activity of wild strawberry grown under different salinity levels (32.5, 50 and 100 mM L^{-1} NaCl). Assimilatory pigments content, free proline concentration, chlorophyll fluorescence and relative water content were measured at two phenological phases BBCH 15 and 60. Results analysis revealed that the applicability of Tytanit® to mitigate physiological stress in wild strawberry caused by salinity did not produce the desired effect.

Key words: chlorophyll-a, chlorophyll-b, carotenoids, proline, chlorophyll fluorescence, water balance.

INTRODUCTION

In research on the influence of unfavorable environmental factors on physiological activity of plants, solutions are sought to optimize plants growth and development conditions. The most frequently applied solution is utilization of natural or mineral fertilizers, growth regulators or protective preparations. Tytanit® is a trade name for a liquid, mineral plant growth and yield stimulator the main component of which is titanium (Ti). The beneficial effect of Ti has been confirmed by a number of scientific reports revealing its influence on the increase in the activity of iron ions, vigour of pollen grains, rate of nutrient uptake, and the improvement of plant health status (Michalski, 2008; Borkowski et al., 2017). Freshwater scarcity, environmental pollution and salinisation of soil and water are significant problems at global level. According to Jamil et al. (2011) and Shrivastava and Kumar (2015) high salinity afflicts about 20% of total crops and 33% of irrigated agricultural lands worldwide. Further predictions indicate that with the increase of salinized areas at 10% rate annually over 50% of the arable land would be salinized by the year 2050.

Salinity causes a decrease in cultivated area, yield and quality of crops (Yamaguchi et al., 2005; Shahbaz et al., 2013). More than half of the crop species is sensitive to even relatively low salinity. Excess salt in the root zone lowers the amount of water available to plants (Zhu, 2001). Plants exposed to prolonged salinity suffer from ionic stress which usually appears as premature ageing of leaves. This results in a reduction in the total photosynthetic area and thus plant productivity (Sultana et al., 1999; Muscolo et al., 2003).

Wild strawberry (*Fragaria vesca* L.) belongs to the family Rosaceae. *F. vesca* is mostly known for its aromatic fruits, however, almost whole plant has medicinal or therapeutic properties. Leaves and stems contain important antioxidant agents such as silicium acids, quercetin and flavonoids. Fruits are rich in vitamins C, A, B, and mineral salts: iron, calcium, phosphorus and cobalt (Yurdugul, 2008). High antioxidant content makes the species beneficial for human health. Enriching daily diet in wild strawberry prevents premature ageing, supports the immune system, helps control: diabetes, high blood pressure, high cholesterol, and lowers the risk of cancer. Nevertheless, due to their

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specific taste and intense aroma and high antioxidant content, wild strawberries are valued and sought by the fresh fruit markets, the processing industry, confectionery and cosmetics industries. That contributes to a high market value of *Fragaria vesca*, which may encourage farmers to increase the cultivation area of this species (Muñoz et al., 2011; Oancea, 2011; Dias et al., 2016; Kruczek et al. 2020).

The literature so far has focused mainly on the evaluation of the influence of Tytanit® on the yield and its quality in various plant species. There are few reports investigating the issue of its protective properties based on physiological mechanisms observed in plants. Most of this type of research is carried out on popular cultivated plants (3c). There are few reports concerning niche species, such as wild strawberry (*Fragaria vesca* L.), which becomes increasingly popular in Poland due to its taste and biological properties.

The aim of the experiment was to investigate an influence and effectiveness of Tytanit® on the physiological activity of wild strawberry of Baron von Solemacher variety cultivated in saline conditions.

MATERIAL AND METHODS

Plant material and experimental design

The vase experiment was carried out in 2016-2017 in the period from April to September in the vegetation hall of the West Pomeranian University of Technology in Szczecin. The experimental material was the wild strawberry *Fragaria vesca* L. var. Baron von Solemacher, grown in a medium with three levels of salinity in the presence or absence of Tytanit®.

The wild strawberry variety tested is characterized by medium tolerance to salinity and high yield. The variety grows up to 20 cm high, has elongated red or cream-colored fruit, rich in vitamins B and mineral salts: iron, calcium, phosphorus, cobalt.

Each year, wild strawberry seeds were sown into a ready-made garden substrate on the basis of peat and sand. The substrate had pH 6, salinity 32.5 mM L^{-1} NaCl and starting dose of multi-compound fertilizer with the composition of NPK 14+16+18 in the amount of 0.6 kg m^{-3}. After 6 weeks, young seedlings were moved into vases with the volume of 1 dm^3 and placed in a vegetation hall. Planted seedlings of wild strawberry were daily irrigated with distillate water up to minimal seepage of the substrate. The substrate moisture was maintained within the range of 2.2 - 1.7 pF.

The vase experiment was established in the system of random blocks in three repetitions of 8 plants each. The first factor was the 3-degrees of substrate salinity: S1 - ready-made substrate salinity 32.5 mM L^{-1} NaCl; S2 - 50 mM L^{-1} NaCl; S3 - 100 mM L^{-1} NaCl, The second factor was the use of Tytanit® (T) - 8.5 g of titanium per liter of preparation - in one dose at all salinity levels: S1+T, S2+T, S3+T. The substrate was watered with a solution of 0.3% concentration (30 ml of stimulator in 100 L water) in three stages of plant development after seedlings rooting: 2-3 leaf developed, 5-8 leaf developed and at the beginning of flowering, according to the manufacturer's recommendations. The control was performed on plants cultivated on a substrate with salinity of S1 without treatment with Tytanit®. The measurements of selected parameters were performed in two phenological phases of BBCH 15 (5th leaf developed) and BBCH 60 (first flowers open). The material for chemical analyses consisted of leaves taken from the plant rosette center. Moreover, after the experiment, the substrate analysis was performed to determine the changes in macroelements content and salinity.

Assimilatory Pigments

A fresh leaves of *F. vesca* were used to determine the content of assimilatory pigments: chlorophyll-a and chlorophyll-b by the Arnon's method (1956) as modified by Lichtenthaler and Wellburn (1983). The content of carotenoids was assayed according to the Hager and Mayer-Berthenrath's (1966) method. The leaves were homogenised with 10 ml of 80% acetone and the optical density of the obtained supernatants was determine on a spectrophotometer at 440, 645, and 663 nm wavelengths.

Chlorophyll fluorescence

Fluorescence of chloropchyll-a was measured with the Hanstech Intrument Handy PEA fluorimeter on completely darkened leaves and after light exposure. Two parameters were selected to evaluate the reaction of plants to salinity and Tytanit®: Fv/Fm - maximum potential photochemical activity and Fv/Fo - maximum water fission efficiency on the donor side of PSII.

Proline (Pro) determination

The concentration of free proline in fresh wild strawberry leaves was determined using the ninhydrin reaction according to the method developed by Bates et al. (1973). 0.5 g of fresh

plant tissue was homogenized in the presence of 3% salicylic acid aqueous solution (10 ml) and the homogenate was filtered through a tissue paper filter. 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added to the upper aqueous phase. Then the whole was mixed and placed in a closed tube in a greenhouse set at 90-100°C. After 1 hour, the tubes were transferred to an ice bath for 15 minutes to cool. Then 4 ml of toluene was added to each tube and shaken for 30 min. The samples prepared in this way were left for phase separation. The upper phase (toluene) was collected to determine the absorbance of chromophore in the presence of a blank at a wavelength of $\lambda = 520$ nm (Auriga et al. 2018).

Water balance

The water balance in wild strawberry leaves was determined by calculating the following indicators: RWC – relative water content, and WSD – water saturation deficit (Yamasaki and Dillenbu 1999) according to the following equations: RWC = [(fresh weight– dry weight) / (turgid weight – dry weight)] x 100; WSD = [(turgid weight – dry weight) - (fresh weight – dry weight) / (turgid weight – dry weight)] x 100. The procedure was performed according to the method described by Wróbel et al. (2016).

Statistics

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

RESULTS AND DISCUSSION

The results on the susbstrate analysis are presented in Table 1. The results showed the effect of Tytanit® on the increase in the content of elements K, Ca, Mg, Na in the medium with the lowest salinity compared to the variant without using the preparation. In substrates with higher salinity in which Tytanit® was used, lower values were noted for all nutrients except nitrogen in the S3 + T variant. The content of this element was the highest among the tested variants (95 mg l^{-1}).

Chlorophylls and carotenoids

The highest coefficient of an influence on chlorophyll-a and carotenoids content was noted for salinity, P=32.07% and P=30.70%, respectively. The interactions between salinity and date P=24.17% and P=29.27% and between Tytanit® use and salinity P=15.10% and P=16.08% were also significant (Tab. 2). The most significant influence on chlorophyll-b content was found for salinity P=27.34%, lower interaction between the use of Tytanit® and the term P=20.94% and the use of Tytanit® alone P=12.27%. Among the studied factors, the lowest influence on the content of assimilatory pigments was observed for the time of measurement.

In the first period of measurement, regardless of the substrate salinity level, no significant effect of Tytanit® on the content of individual assimilatory pigments was observed. Although the differences were insignificant, more of them were found in S1+T objects (Tab. 3). In BBCH 60 was observed a 2-3-fold decrease in pigments content after an application of the preparation in the highest salinity conditions. In general, the higher the salinity, the larger the decrease in pigments content. However, Tytanit® significantly increased the concentration of chlorophyll-a and b and carotenoids in plants grown on substrate S1. Their content in the first and second period of measurement was as follows: chl a 1.921 and 2.185 mg kg^{-1}; chl b 0.700 and 0.783 mg kg^{-1}; carotenoids 4.008 and 5.014 mg kg^{-1}, respectively.

Table 1. Analysis of the substrate.

Variant	pH in H2O	mg l^{-1}							Salinity in g NaCl l^{-1}
		N-NO3	P	K	Ca	Mg	Na	Cl	
S1	6.0	72	43	327	1923	200	259	170	1.91
S2	6.1	50	47	352	2047	224	1802	2260	5.51
S3	6.0	72	44	378	1898	200	2387	4450	10.86
S1 + T	6.2	64	41	366	2118	244	308	189	1.90
S2 +T	6.0	68	44	325	2038	222	1600	1940	5.97
S3 +T	6.0	95	43	383	1918	211	2347	4070	10.23

S1 – substrate with slinity 32.5 mM L^{-1}; S2 - 50 mM L^{-1}; S3 – 100 mM L^{-1}; T – 0.3% solution of Tytanit®

Table 2. ANOVA table for pigments content, proline content, Fv/Fm and Fv/Fo.

	Chl-a		Chl-b		Carotenoids		Pro		Fv/Fm		Fv/Fo	
Source of variance	p	P(%)	p	P(%)	p	P(%)	p	P(%)	p	P(%)	p	P(%)
a	0,000	**5.56**	0.000	**12.27**	0.000	**4.34**	0.000	**0.53**	0.910	**0.00**	0.765	**0.03**
b	0,000	**32.07**	0.000	**27.40**	0.000	**30.70**	0.000	**56.84**	0.000	**25.09**	0.000	**26.54**
c	0,000	**4.26**	0.618	**0.13**	0.040	**1.24**	0.000	**28.93**	0.000	**37.28**	0.000	**25.88**
a×b	0,000	**15.10**	0.007	**6.26**	0.000	**16.08**	0.000	**1.78**	0.084	**0.19**	0.247	**0.48**
a×c	0,000	**8.34**	0.000	**20.94**	0.000	**7.32**	0.022	**0.16**	0.224	**0.19**	0.375	**0.70**
b×c	0,000	**24.17**	0.001	**10.02**	0.000	**29.27**	0.000	**11.08**	0.000	**32.04**	0.000	**17.11**
a×b×c	0,002	**4.39**	0.000	**10.92**	0.001	**4.73**	0.410	**0.05**	0.342	**0.14**	0.234	**1.04**
Error		6.11		12.05		6.30		0.63		5.07		28.21
Total		100.0		100.0		100.0		100.0		100.0		100.0

a – Tytanit®; b – salinity; c – phenological phase; p – probability of error; P (%) – percentage of contribution

Tab. 3. Effect of the performed treatments on pigments content (mg g^{-1} f.w.) and free proline content (µmol g^{-1} f.w.) in the leaves of the tested wild strawberry. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

Salinity	BBCH15		**average**	BBCH60		**average**
	0	T		0	T	
			Chlorophyll-a (mg g^{-1} f.w.)			
S1	1.651^{bcd}	1.921^{ab}	**1.786^{B}**	2.007^{ab}	2.185^{a}	**2.096^{A}**
S2	1.777^{bc}	1.736^{bcd}	**1.756^{B}**	1.852^{abc}	1.396^{d}	**1.624^{B}**
S3	1.758^{bcd}	1.663^{bcd}	**1.710^{B}**	1.535^{cd}	0.497^{e}	**1.016^{C}**
average	**1.729^{A}**	**1.773^{A}**		**1.797^{A}**	**1.359^{B}**	
			Chlorophyll-b (mg g^{-1} f.w.)			
S1	0.605^{c}	0.700^{abc}	**0.653^{B}**	0.836^{a}	0.783^{ab}	**0.810^{A}**
S2	0.666^{bc}	0.695^{abc}	**0.681^{B}**	0.780^{ab}	0.611^{c}	**0.695^{B}**
S3	0.699^{abc}	0.661^{bc}	**0.680^{B}**	0.748^{abc}	0.326^{d}	**0.537^{C}**
average	**0.657^{B}**	**0.685^{B}**		**0.788^{A}**	**0.573^{C}**	
			Carotenoids (mg g^{-1} f.w.)			
S1	3.445^{cde}	4.008^{bcd}	**3.727^{B}**	4.387^{ab}	5.014^{a}	**4.700^{A}**
S2	3.720^{bcde}	3.777^{bcde}	**3.74^{B}**	4.219^{abc}	3.078^{e}	**3.650^{B}**
S3	3.836^{bcde}	3.564^{bcde}	**3.700^{B}**	3.289^{de}	1.111^{f}	**2.200^{C}**
average	**3.667^{A}**	**3.783^{A}**		**3.965^{A}**	**3.068^{B}**	
			Proline (µmol g^{-1} f.w.)			
S1	0.585^{f}	0.283^{f}	**0.434^{D}**	1.048^{e}	1.108^{e}	**1.078^{C}**
S2	0.445^{f}	0.527^{f}	**0.486^{D}**	1.415^{de}	1.752^{cd}	**1.583^{B}**
S3	2.100^{c}	1.365^{de}	**1.733^{B}**	4.905^{a}	4.229^{b}	**4.567^{A}**
average	**1.044^{B}**	**0.725^{C}**		**2.456^{A}**	**2.363^{A}**	

S1 – substrate with slinity 32.5 mM L^{-1}; S2 - 50 mM L^{-1}; S3 – 100 mM L^{-1}; 0 – lack of Tytanit®; T – 0.3% solution of Tytanit®

The study showed a significant decrease in the mean content of assimilatory pigments caused by salinity of the substrate in later phenological phases (II term). This confirms the literature reports on the plant reactions to this factor (Sahat et al., 2010; Chutipaijit et al., 2011; Parihar et al., 2015). Rahimi et al. (2011) in the experiment with strawberry (*Fragaria ananassa cv. Camarosa*) showed a decrease in chlorophyll-a content depending on the degree of substrate salinity, by about 15% for 60 mM L^{-1} NaCl and 36% for 90 mM L^{-1} NaCl compared to the control. In the case of chlorophyll-b it was about 26% and 41%, respectively. In our study, a decrease of about 8% and 24% for chlorophyll-a and about 7% and 10.5% for chlorophyll-b was noted for salinity of 50 and 100 mM L^{-1} NaCl. This may suggest better salinity resistance of wild strawberry compared to strawberry. Changes in the content of photo-synthetic pigments are also dependent on the tolerance of the plants to salinity of the substrate, i.e., their genotype (Garcı, 2002; Noreen et al., 2009). On the other hand, a positive effect of salinity on the content of assimilatory pigments was noted in studies on rice (Belkhodja et al., 1999) and tomato (Romero-Aranda et al., 2001).

Similar results were obtained in our experiment, but only in the first phenological phases (first term of measurement). An increase in the content of assimilatory pigments under the influence of salinity in the first period of ontogenesis was associated with a decrease in hydration of leaf assimilating tissue, and thus with a higher concentration of pigments per mass unit (Wróbel et al., 2016).

In turn, the observed significant decrease in assimilatory pigments under the influence of salinity in later phenological phases (II term) might be associated with a decrease in metabolic activity of the organism. It could be result from strong dehydration of cells, and thus decreased synthesis of chlorophyll and carotenoids. Lowest pigments values recorded at BBCH 60 for plants S3+T may indicate an unfavorable effect of Tytanit® on plants cultivated under extreme conditions. The very low pigments content compare to other variants suggests a decrease in photosynthesis and may designate preparation's enhancement of the toxic effect of salt on the plant.

Significant positive effect of Tytanit® on physiological-biochemical activity of wild strawberry was observed only in unsalted substrate, regardless of the phenological phase. Positive effect of titanium dioxide on chlorophyll-a,-b and carotenoids content was confirmed in many publications(Carvajal et al. 1998; Hrubý et al. 2002; Samadi, 2014; Samadi et al., 2015). According to Kuzel et al. 2003, titanium causes apparent deficiency of Fe and Mg, contributing to a greater absorption of Fe and other metals by roots. Iron, on the other hand, is crucial for chlorophyll formation and photosynthesis rate (Nadi et al., 2013). It also has an important effect on enzymatic systems and plant respiration (Mir et al., 2015). In turn, an increased carotenoid content may be the result of an increased synthesis of this pigment caused by suppression of reactive oxygen species by heavy metals (Samadi, 2014).

Proline

The research revealed a significant influence of all studied factors on proline content in wild strawberry leaves (Tab. 2). The salinity P=56.84%, and the term P=28.93% had the most significant effect, while the lowest was noted for Tytanit® P=0.53%.

Plants cultivated under conditions of the highest salinity were characterized by a considerably high amount of proline regardless of the date of measurement (Tab.3). In general, older plants (BBCH 60) had significantly higher proline content compared to younger plants (BBCH 15). Tytanit® treatment significantly decreased proline accumulation only in plants in BBCH 15 – variants with T absence 1.044 and variants with T presence 0.725 µmol g^{-1} f.w. – and had no significant effect on older plants.

Proline is an enzyme which main function in the plant is to stabilize proteins and protect cell membranes. It also acts as an osmoprotector and is a reservoir of nutrients, mainly carbon and nitrogen (Islam et al., 2009; Zouari et al. 2016). It is considered to be one of the main salt stress biomarkers (Gomes et al., 2017). Its content in the plant depends on many biotic and abiotic factors (Karolewski 1996).

Rahimi et al. (2011) recorded a fourfold increase in proline content in strawberry leaves in case of substrate salinity of 60 mM L^{-1} and a fivefold increase for salinity of 90 mM L^{-1}. In our experiment the concentration of Pro slightly increased at salinity of 50 mM L^{-1}, whereas at salinity of 100 mM L^{-1} the result was four times higher compared to the control. This may indicate a different level of both species reaction to salt stress. Plants treated with Tytanit® were characterized in both terms by a lower mean Pro content compared to untreated plants, which may indicate the anti-stress effect of this preparation. However, there are no reports on this subject in the literature.

RWC and WSD

Statistical analysis showed a significant influence of salinity and time of measurement on the decrease of relative water content in wild strawberry leaves. Salinity had a much greater effect than the term. In BBCH 15, plants were characterized by a smaller water deficit in leaves. On the other hand, Tytanit® differentiated the RWC and WSD indices in the studied phases, but in an insignificant way – Table 4.

Significant decrease of relative water content in wild strawberry leaves in the second period of measurement at salinity S2 and S3 indicate a high accumulation of salts in the substrate. Soil salinity inhibits plant growth processes, reduces physiological activity and leads to physiological drought deteriorating the water balance of cells (Wróbel et al., 2016).

Moaveni et al. (2011) observed a positive effect of titanium on the relative water content in plant tissue in studies with wheat grown under optimal conditions. The lack of significant influence of this element on the water balance of wild strawberries in this experiment may prove that the action of Tytanit® may depend on the type of stress factor.

This preparation may also have a different effect on individual plant species.

Fluorimetric analysis

In the experiment with the wild strawberry, the maximum potential photochemical yield of PSII (Fv/F_M) depended mostly on the date of measurement P=37.28%, then on the interaction between salinity and date P=32.04% and on the salinity itself P=25.09% (Tab. 2).

The mean value of Fv/F_M was significantly lower in the second term of measurement regardless of the use of Tytanit® (Tab. 5). The highest salinity of the substrate caused a significant decrease in the value of this parameter in the second term of measurement, and the value of the maximum quantum yield of PSII was almost thirty times lower than the value measured in the first term.

The salinity P=26.54%, followed by the measurement date P=25.88% and the interaction between these factors P=17.11% had the most significant effect on the maximum water fission efficiency on the donor side of PSII (Fv/Fo). The error value (P=28.21%), similarly as in the case of carbon dioxide assimilation, exceeded the share of the examined factors; Tytanit® application, term and salinity. This indicates a greater influence of factors not studied in the experiment on the value of Fv/Fo in wild strawberry. The mean value of this parameter was significantly lower in the second term of measurement regardless of the use of Tytanit® (Tab. 5). The highest salinity of the substrate caused a significant decrease in the parameter in BBCH 60, the mean value of Fv/Fo was almost two hundred times lower than the mean value of this parameter measured in BBCH 15.

Chlorophyll fluorescence is used in eco-physiological studies, monitoring of crops and ecosystems threatened by phytotoxic factors and in plant tolerance to various stress factors (Kalaji et al., 2010). Fluorescence is a highly sensitive photosynthetic plant retraction test that detects changes in the overall bioenergy status of a plant (Schweiger et al. 1996; Michałek et al., 2005).

Table 4. Effect of the performed treatments on ralative water content and water saturation deficit in wild strawberry leaves. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

Salinity	BBCH15		**average**	BBCH60		**average**
	0	T		0	T	
			RWC (%)			
S1	79.04^{a}	73.50^{a}	**76.27^{A}**	72.83^{a}	68.75^{ab}	**70.79^{A}**
S2	76.28^{a}	73,61^{a}	**74.95^{A}**	44.66^{cd}	54.81^{bc}	**49.74^{B}**
S3	65.98^{ab}	71.30^{ab}	**68.64^{A}**	37.67^{cd}	31.21^{d}	**34.44^{C}**
average	**73.77^{A}**	**72.80^{A}**		**51.72^{B}**	**51.59^{B}**	
			WSD (%)			
S1	20.96^{d}	26.50^{d}	**23.73^{C}**	27.17^{d}	31.25^{cd}	**29.21^{C}**
S2	23.72^{d}	26.39^{d}	**25.06^{C}**	55.34^{ab}	45.19^{bc}	**50.27^{B}**
S3	34.02^{cd}	28.70^{cd}	**31.36^{C}**	62.33^{ab}	68.79^{a}	**65.56^{A}**
average	**26.23^{B}**	**27.20^{B}**		**48.28^{A}**	**48.41^{A}**	

S1 – substrate with slinity 32.5 mM L^{-1}; S2 - 50 mM L^{-1}; S3 – 100 mM L^{-1}; 0 – lack of Tytanit®; T – 0.3% solution of Tytanit®

Table. 5. Effect of the performed treatments on chlorophyll fluorescence parameters in the leaves of the tested wild strawberry. Means with same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

Salinity	BBCH15		**average**	BBCH60		**average**
	0	T		0	T	
			Fv/Fm			
S1	0.750^{a}	0.721^{a}	**0.735^{A}**	0.691^{a}	0.667^{a}	**0.679^{A}**
S2	0.740^{a}	0.723^{a}	**0.731^{A}**	0.643^{a}	0.723^{a}	**0.683^{A}**
S3	0.716^{a}	0.699^{a}	**0.708^{A}**	0.006^{b}	0.025^{b}	**0.014^{B}**
average	**0.735^{A}**	**0.714^{A}**		**0.436^{C}**	**0.505^{B}**	
			Fv/Fo			
S1	3.012^{a}	2.785^{a}	**2.898^{A}**	2.500^{a}	2.157^{a}	**2.328^{A}**
S2	2.944^{a}	2.645^{a}	**2.794^{A}**	1.985^{a}	2.669^{a}	**2.327^{A}**
S3	2.650^{a}	2.562^{a}	**2.606^{A}**	0.006^{b}	0.027^{b}	**0.015^{B}**
average	**2.868^{A}**	**2.664^{A}**		**1.453^{B}**	**1.743^{B}**	

S1 – substrate with slinity 32.5 mM L^{-1}; S2 - 50 mM L^{-1}; S3 – 100 mM L^{-1}; 0 – lack of Tytanit®; T – 0.3% solution of Tytanit®

According to Angelini et al. (2001), the maximum photochemical yield of PSII (Fv/F_M) is a reliable indicator of the photochemical activity of photosynthetic apparatus. For the majority of plants at the stage of full development and under optimal conditions, the value of this parameter is about 0.83. In the case of our experiment with wild strawberry, the Fv/F_M value for the control was slightly lower, about 0.74. In turn, the decrease in this parameter value indicates that the plant was exposed to stress factors that damaged PSII functions, reducing the efficiency of electron transport. A significant decrease in Fv/F_M values was observed in our experiment with wild strawberries as a result of salt stress.

Michałek and Sawicka (2005) in the experiment with different potato cultivars confirmed the dependence of Fv/F_M parameter on the phenological phase of the plant and also on the cultivar. The authors obtained a higher Fv/F_M value for the majority of the cultivars studied at full emergence than at full flowering. Similar results were obtained in our study with wild strawberry. Rahimi et al. (2011) recorded a significant decrease in Fv/F_M value as a result of salt activity in strawberry physiology. The authors stated that the decreased quantum yield may result from the structural influence of salinity on PSII. Salinity affected reactive photo-system centers directly or through aging of the cells. Salt stress is conducive to leaf aging. NaCl induces a decrease in the concentration of protein and chlorophyll, which was noted already in 1987 by Kura-Hotta et al. A decrease in the value of maximum water fission efficiency on the donor side of PSII indicates the damage to the photosystem (Xiaoqing et al. 2004).

In plants growing in salinity conditions, the value of Fv/Fo ratio decreases, which indicates a decrease in the efficiency of water fission reaction and weakening of photosynthetic electron transport (Pereira et al. 2000). The use of Tytanit® did not have a significant effect on both parameters of photochemical activity (Tab. 2). A lower Fv/Fo value recorded in plants treated with Tytanit® in the first term compared to the control may indicate a negative effect of the preparation on the photosynthetic system. Pereira et al. (2000) showed a decrease in net photosynthesis on citrus as a result of the action of aluminum, which damaged the photosynthetic apparatus. According to the authors, a decrease in Fv/Fo coefficient is an indicator of structural damage that occurs in tylakoids and affects the photosynthetic transport of electrons.

Higher mean values of both Fm/Fv and Fv/Fo parameters in the second term of measurement in plants treated with Tytanit® may be a reflection of weak support of plant defense mechanisms.

CONCLUSIONS

The results of the experiment showed a varied effect of Tytanit® on the physiological and biochemical features of wild strawberry grown in salinity.

In general, Tytanit® treatment caused a significant decrease in assimilatory pigments content in older plants and did not affect plants in BBCH 15. On the other hand, preparation significantly decreased proline content in young plants but overall did not affect proline level in older plants. Moreover, Tytanit® had no significant effect on water balance improvement both, in the case of medium salinity and control variants. However, at highest salinity, it deteriorated the water management of plants, increasing the water deficit in the leaves.

The measured parameters of chlorophyll-a fluorescence: Fv/FM and Fv/Fo proved to be adequate in diagnosing the occurrence of salt stress in wild strawberry. A significant decrease in these values has been demonstrated for saline soil.

The applicability of Tytanit® to mitigate physiological stress in wild strawberry caused by salinity did not produce the desired effect.

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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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