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TABLE OF CONTENTS

Agriculture

Animal Breeding and Husbandry

Biology

Food and Nutrition Sciences

Environmental Protection

Humans and Environment

SPIS TREŚCI

Rolnictwo

Chów i hodowla zwierząt

Biologia

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EFFECTIVENESS OF *RHIZOBIUM* INOCULATION ON PRODUCTIVITY OF COMMON BEAN (*PHASEOLUS VULGARIS* L.): INVESTIGATING THE EFFECT OF INDIGENOUS RHIZOBIA POPULATION

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K e y w o r d s: Common bean, *Rhizobium*, indigenous rhizobia population.

A b s t r a c t

This study was conducted to evaluate the effect of rhizobial population on the effectiveness of locally isolated elite isolates of *Rhizobium* on common bean at the major growing area of Eastern Ethiopia. The result showed significant effect of inoculation, the varieties and their interaction on nodulation, yield and yield traits, except for the number of seed per pod. Most of tested *Rhizobium* isolates significantly improved the nodule number and nodules dry weight in all soils regardless of rhizobial population. Significant increase in total biomass yield and grain yield of common bean was recorded with NSCBR-14, inorganic N-fertilized and NSCBR-$(25)_2$ treatments in soil with a high, low and moderate rhizobial population, respectively. The highest values of most of the yield traits including NN and NDW in all experimental sites was recorded with Dursitu variety but the highest values GY and TBY with Kufanzik. Hence, the indigenous rhizobial population did not affect the effectiveness of inoculation but the soil types and varieties affect the effectiveness of the isolates.

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WPŁYW INOKULACJI *RHIZOBIUM* NA PRODUKCJNOŚĆ FASOLI ZWYCZAJNEJ (*PHASEOLUS VULGARIS* L.). POSZUKIWANIE WPŁYWU RDZENNYCH POPULACJI *RHIZOBIUM* NA TEN CZYNNIK

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S ł o w a k l u c z o w e: fasola zwyczajna, *Rhizobium*, rdzenna populacja rhizobia.

A b s t r a k t

Badania przeprowadzono w celu określenia wpływu populacji *Rhizobium*, lokalnie wyizolowanych elitarnych izolatów *Rhizobium*, na produkcyjność fasoli zwyczajnej w głównym obszarze upraw wschodniej Etiopii. Uzyskane wyniki wskazują na istotny wpływ szczepienia odmian i ich interakcji na wykształcenie brodawek korzeniowych, plon i jego cechy, z wyjątkiem liczby nasion w strąku. Większość badanych izolatów *Rhizobium* istotnie zwiększyła liczbę brodawek i suchą masę brodawek we wszystkich glebach, niezależnie od populacji *Rhizobium*. Istotny wzrost ogólnego plonu biomasy i plonu nasion fasoli zwyczajnej zaobserwowano odpowiednio z użyciem NSCBR-14, nieorganicznego nawożenia azotem i NSCBR-$(25)_2$ w glebie z dużą, małą i średnią populacją *Rhizobium*. Najwyższe wartości większości cech wydajności plonu, w tym NN i NDW we wszystkich miejscach badań, wykazano dla odmiany Dursitu, a najwyższe wartości GY i TBY stwierdzono dla odmiany Kufanzik. W związku z tym rdzenna populacja *Rhizobium* nie wpływała na efektywność inokulacji, ale typy gleb i odmiany działały na skuteczność izolatów.

Introduction

Common bean (*Phaseolus vulgaris* L.) is widely cultivated in Central and South America and in many countries of Africa and Asia and serves as the main source of protein for human consumption. Common beans are generally grown in mixed stands with maize or are planted to fill up spaces between other crops; monocrop beans are rare in the eastern part of Ethiopia. It occupies more than 323,317.99 hectares with annual production of 513,724,81 ton in Ethiopia, but the average yield of beans is often low (1.55 ton ha^{-1}) (CSA 2015). However, the yield potential as obtained in research stations is normally well over 2 tons per ha (MEKBIB 2003). These low yields could, to a large extent, be attributed to poor soil fertility or other soil-related constraints (WORTMANN et al. 1998). Of which soil N availability is the most limiting nutrient crop production in Ethiopia (KARLTUN et al. 2013). Biological nitrogen fixation (BNF) is one of the main sources of N by which N_2 derived from atmospheric N_2 to soil N. It has economic, environmental, and agronomic benefits and could be also as an alternative to synthetic fertilizers.

The rhizobial species nodulating common bean are *Rhizobium etli*, *Rhizobium leguminosarum* bv. *phaseoli*, *Rhizobium gallicum* bv. *phaseoli*, *Rhizobium giardinii* bv. *phaseoli,* and *Rhizobium tropici* (MARTÍNEZ-ROMERO 2003). Several studies showed the promising potential of common bean to fix nitrogen (GARCÍA et al. 2004, REMANS et al. 2008). Inoculation of local or adapted varieties of common bean with effective native strains produced similar grain yield with those treated inorganic N fertilizer (HUNGRÍA et al. 2000, 2003, MOSTASSO et al. 2002, MRABET et al. 2005).

Due to promiscuous nature of common bean, poor nodulation and variable response to inoculation were recorded (MICHIELS et al. 1998). The presence of well-established rhizobial population due to prior cropping of legumes is also one of the principal limitation for inoculation success (SADOWSKY and GRAHAM 1998, SESSITSCH et al. 2002). Naturalized or indigenous rhizobia in the soil have been shown to reduce the effectiveness of inoculated rhizobial strains by competing for infection sites and ultimately nodulating the host plant (ZENG et al. 2007). Unfortunately, the majority of common bean growing soils of Ethiopia contain high numbers of indigenous rhizobia which are often ineffective in symbiosis but highly competitive due to their adaptation to given environmental conditions (ARGAW 2015, 2016). ELIAS and HERRIDGE (2015) found that nodules occupancy by inoculated *Rhizobium* declined by an average 17% with each log unit increase in numbers of native chickpea rhizobia.

The success of inoculation requires that the inoculated isolate must be both highly effective in N2 fixation and highly competitive against the native isolates in the soil (WILLIAMS and PHILLIPS 1983, SINGLETON and TAVARES 1986, SEGOVIA et al. 1991). Most efficient and competitive rhizobia can also increase the symbiotic efficiency of leguminous plants in soils containing native rhizobia (CHEMING'WA and VESSEY 2006, SESSITSCH et al. 1998). The suitability of native strains as inoculants of local common bean has been previously demonstrated for *Rhizobium tropici* in America (HUNGRÍA et al. 2000, 2003, MOSTASSO et al. 2002), and for *Rhizobium gallicum* in Africa (MRABET et al. 2005). MEADE and O'GARA (1985) suggested the use of environmentally adapted indigenous rhizobia strains as inoculants in soils containing indigenous rhizobial populations.

Most African countries, including Ethiopia, have not exploited the benefits of rhizobia inoculation technology. Therefore, it is important to characterize the indigenous population, to understand responses, in soils with different indigenous rhizobial population nodulating common bean. The response of bean to *Rhizobium* inoculation is indeed likely to be influenced by rhizobia strain, bean cultivar, and climatic variables. The objective of this work was to evaluate the effectiveness of elite rhizobia isolates-improved common bean varieties symbiosis across naturalized common bean rhizobia population gradients.

Materials and Methods

Experimental sites

Field sites were selected to cover a range of soil types with varied rhizobial population nodulating common bean ranging from <100 to >1000 rhizobia g^{-1} soil in Eastern Ethiopia, where common bean is cultivated solely and intercropped with sorghum and maize without inoculation, as previous research had shown that rhizobial population can affect the response different to *Rhizobium* inoculation and pelleting treatments (THIES et al. 1991). Accordingly, the four experimental sites namely: Haramaya, Hirna, Fedis, and Babillae were selected.

Field experiments were conducted in four sites which are Hirna (09°13.157'N and 041°06.488'E at an altitude of 5932 ft above sea level [asl]), Fedis (09°06.941'N and 042°04.835'E at an altitude of 5476 ftasl), Babillae (09°13.234'N and 042°19.407'E at 5478 ft asl) and Haramaya (09°24.954'N and 042°02.037'E at an altitude of 6628 ft asl) agricultural research centers in 2012, representing the major common bean cultivating areas of Ethiopia. The initial soil samples for soil physic-chemical were collected from the top 0–20 cm. A composite soil comprising at least 20 point samples was transported back to the laboratory within a day. Representative subsamples of 1 kg each were prepared for most probable number (MPN) assay and standard analyses. For MPN experiment, the soil sample was stored in a cold room (4°C) until used in a pot experiment evaluating the symbiotic potential of indigenous rhizobia. The soil physic-chemical properties were analyzed using standard procedure (SAHILEMEDIN and TAYE 2000) and presented in ARGAW et al. (2015).

Enumeration of indigenous Rhizobium population nodulating common bean

The initial indigenous rhizobia population nodulating common bean was determined by the plant infection technique, using dilution of soil for nodulation tests according to the method of VINCENT (1970). Measured amounts of 30 cm^3 sterilized N-free nutrient solution were placed in growth pouches. This experiment was conducted moisture and light controlled growth chamber. One cm^3 of soil dilution of 10^{-1} to 10^{-10} was added to the sterile sand culture in 250 cm^3 capacity pot. Each dilution was replicated four times. Sterilized common bean seeds were placed in each pot. After 3 weeks, the presence or absence of nodules on the common bean roots was recorded for each dilution. The MPN was calculated from the most likely number, using the MPN tables.

Source of the isolate and seed variety

Eight isolates of *Rhizobium* spp. for this study were obtained from Biofertilizer research and production project, Haramaya University (Haramaya, Ethiopia) and designated as HUCBR-1, HUCBR-2, HUCBR-3, HUCBR-4, HUCBR-5, HUCBR-6, HUCBR-7, and HUCBR-8. All strains used in this study were isolated from Ethiopia soils. All are characterized as superior isolates in N_2 fixation potential under greenhouse condition (ARGAW 2016).

Seeds of *Phaseolus vulgaris* varieties used in this study were obtained from Lowland Pulse Research Program, Haramaya University, Haramaya, Ethiopia. Varieties were selected based on their yield, their maturity time, and improved recently released Varieties of the experimental region. The selected varieties were Gofta, Kufanzik and Dursitu identified as early, moderate and late maturing variety, respectively.

Preparation of inoculums for the experiments

The pure culture of *Rhizobium* strains was obtained from the laboratory in slant culture. The bacteria were cultured in YEM (Yeast extract mannitol) agar medium and then single pure colony was transferred into YEM broth medium and kept at 30°C for 7 days on a rotary shaker at 120 rpm. The *Rhizobium* sp. culture liquid medium with 400 cm^3 was added to 1 kg of the carrier (sterile fine filter mud) and mixed thoroughly and then packed in plastic bags. Filer-mud-base inoculum was incubated at 26–28°C for 15 days. At the time of inoculation, the number of rhizobia in the inoculum was estimated by the plate count method. To count the rhizobia in the inoculum, 1 cm^3 samples of serially diluted inoculum at 10^{-6} dilution were plated in YEM agar. Colonies that developed after incubation at 28°C for 5–7 days were recorded. This test indicated that the number of rhizobia was more than 1×10^9 g^{-1} inocula.

Experimental layout and treatments

The experiments were designed as two-factor experiments in a randomized block design. There were three replications of each treatment. Ten levels of inoculation including eight *Rhizobium* isolates (HUCBR-1, HUCBR-2, HUCBR-3, HUCBR-4, HUCBR-5, HUCBR-6, HUCBR-7, and HUCBR-8) and a non-inoculated control as well as one N-fertilized (20 kg N ha^{-1}) and three common bean varieties were factorially combined. Before sowing, 20 kg P ha^{-1} as trisuperphosphate, and 20 kg N ha^{-1} as urea were applied in furrows

uniformly for all plots. The actual plot was ploughed thoroughly twice with a tractor and divided into sub-plots in accordance with the treatments. The net size of each experimental plot was 3×2 m^2. There were five rows per plot and the spacing was 1.5 m between blocks, 1 m between plots, 40 cm between rows and 10 cm between.

Common bean seeds were sterilized using 70% ethanol for 1 min and NaClO solution (0.25% as available Cl) for 3 min. The seeds were then washed carefully in sterilized deionized water five times before sowing. Then, 20 g of the different rhizobia inoculants was added to different polyethylene bags containing 200 g of common bean seeds. A 10% (w/v) sucrose solution to increase adherence was added to each bag to enhance proper mixing and adhesion of the rhizobia carrier material to the common bean seeds. After mixing, seeds were allowed to air-dry in the shade for 15 min and sown based on the field layout. Two seeds were planted by hand per hole and later thinned down to one per hole 1 week after germination All standard local cultural practices were accomplished throughout the growth period. Manual weeding was also done when required.

Nodulation, yield and yield attributes

At late flowering and early pod setting stage, 5 plants were randomly chosen from central three rows for the evaluation of nodulation and plant growth. The plants were then placed into plastic buckets full of water to loosen the adhering soil. Thereafter, nodules from roots were picked and following data were recorded: (1) Total number of nodules plant^{-1}, and (2) dry weight of nodules plant^{-1}. Shoot dry weight was also measured after the samples were dried at 70°C in the electrical oven until the weight of the samples became constant. The dried shoots were later ground to pass a 0.5 cm sieve. Total N determinations were done by the Kjeldahl method of BREMNER (1965). Yield attributes were determined at full maturity from three central rows. Numbers of pods plant^{-1}, the number of seed pod^{-1}, plant height at harvest and total biomass were evaluated. Grain yield was corrected for 13% moisture content after determining humidity level with a grain moisture tester.

Data analysis

Data were submitted to analysis of variance (SAS INSTITUTE 1999). Statistically significant differences between means were also determined by the LSD test (SAS INSTITUTE 1999).

Results

Nodule number

The rhizobial population nodulating common bean in the study sites were 1.1×10^4, < 100, 2.8×10^3 and 2.5×10^2 in Hirna, Babillae, Haramaya and Fedis soils, respectively. According to HOWIESON and BALLARD (2004), the soil of the study sites was grouped in soils with a high (> 1000 rhizobia g^{-1} soil), moderate (100–1000 rhizobia g^{-1} soil) and low (< 100 rhizobia g^{-1} soil) rhizobial population. Based on this classification, the soils of Babillae, Fedis, and Haramaya and Hirna sites were grouped into a low, moderate and high rhizobia population harboring soils, respectively.

The ANOVA presented that the effect of inoculation treatments, the varieties, and their interaction was significant on nodule number (NN) in all soil types (Table 1). All inoculation treatments except NSCBR-18 resulted in significant increase of NN in soil with a moderate and high rhizobia population (Table 2). In soil with a low rhizobial population, a significant increase in NN was observed in all inoculation treatments excluding NSCBR-57 inoculation.

Among tested varieties, Dursitu recorded significantly higher NN than the remaining varieties in all soil types. Though the effect of N fertilization was non-significant on NN when compared to the control check, a slight reduction of NN was recorded by N application in all soil types. All tested isolates except NSCBR-16 and NSCBR-25 induced significantly ($P < 0.05$) the highest number of nodule in soil containing a high rhizobia population (Figure 1a).

Nodule dry weight

ANOVA showed a significant effect of inoculation treatments, the varieties and their interaction on the nodule dry weight in all soil types except the main effect of varieties on NDW (Table 1). Inoculation of NSCBR-$(25)_2$, NSCBR-16 and NSCBR-25 significant increased the NDW in soil containing a high rhizobia population (Table 2). Ina moderate number of rhizobia populated soil, the NDW was significantly increased by all isolates inoculation except NSCBR-31, NSCBR-18 and NSCBR-57while all isolates significantly improved the NDW in soil with a low rhizobia population.

Among the tested varieties, Dursitu produced significant higher NDW in soils with a high and low rhizobia population.

However, this difference was non-significant in soil containing a moderate rhizobial population. Nevertheless, inorganic N application reduced the NN and the NDW production in soils with a high and moderate rhizobial popula-

Table 1

Summary of ANOVA results for all investigated traits of common bean affected by *Rhizobium* inoculation, varieties, and their interaction, in eastern Ethiopia, 2013/14

Factors	Nodule number			Nodule dry weight [g plant^{-1}]			Shoot dry weight [g plant^{-1}]		
	soil type 1	soil type 2	soil type 3	soil type 1	soil type 2	soil type 3	soil type 1	soil type 2	Soil type 3
Inoculation (I)	11.40***	39.14***	29.79***	6.83***	2.04*	86.39***	4.50***	3.03**	1.96ns
Variety (V)	25.14***	33.68***	16.76***	32.99***	18.62***	1.13ns	17.11***	24.54***	20.77***
I × V	2.34**	16.80***	4.52***	2.51**	5.33***	18.11***	4.11***	3.70***	8.88***
–	number of pods per plant			number of seeds per pod			100 seeds weight		
	soil type 1	soil type 2	soil type 3	soil type 1	soil type 2	soil type 3	soil type 1	soil type 2	Soil type 3
Inoculation (I)	5.25***	5.84***	4.37***	2.65**	0.65ns	2.54*	1.46ns	4.23***	2.49*
Variety (V)	23.19***	12.33***	4.54*	20.90***	22.58***	2.92ns	1143.68***	1448.70***	1729.69***
I × V	1.55ns	5.01***	1.81*	2.19**	0.74ns	1.35ns	0.52ns	4.95***	2.79**
–	total biomass yield			grain yield			total plant N accumulation		
	soil type 1	soil type 2	soil type 3	soil type 1	soil type 2	Soil type 3	Soil type 1	Soil type 2	Soil type 3
Inoculation (I)	3.77***	15.54***	13.88***	9.85***	7.78***	6.58***	0.88ns	12.42***	11.45***
Variety (V)	3.26*	93.49***	206.28***	321.39***	83.77***	159.17***	37.29***	19.43***	248.55***
I × V	1.45ns	10.04***	6.25***	2.24**	11.19***	4.08***	1.09ns	6.25***	8.64***

ns – non significant; * significant at 0.05; **significant at 0.01; ***significant at 0.001; soil type 1 – < 100 rhizobia nodulating common bean g^{-1} soil; soil type 2 – 100–1000 rhizobia nodulating common bean g^{-1} soil and soil type 3 – >1000 rhizobia nodulating common bean g^{-1} soil

Table 2

Nodule number, nodule dry weight and shoot dry weight as influenced by elite *Rhizobium* isolates inoculation and indigenous *Rhizobium* population nodulating common bean, in eastern Ethiopia, 2013/14

Inoculation	Nodule number			Nodule dry weight [g plant^{-1}]			Shoot dry weight [g plant^{-1}]		
	> 1000	< 100	100–1000	> 1000	100–1000	< 100	> 1000	100–1000	< 100
NSCBR-14	189.39^{ab}	99.89^{bc}	143.33^{bc}	0.9468^{abc}	0.1160^{ab}	0.5567^{bc}	63.25^{ab}	42.29^{ab}	54.17^{a}
NSCBR-(25)$_2$	204.06^{ab}	153.33^{a}	159.78^{bc}	1.0306^{ab}	0.1444^{ab}	0.5268^{c}	67.41^{ab}	43.52^{a}	51.70^{a}
NSCBR-59	197.17^{ab}	91.00^{c}	204.44^{a}	0.9081^{abc}	0.1102^{ab}	1.0167^{a}	68.61^{a}	37.32^{abc}	56.76^{a}
NSCBR-31	186.22^{ab}	94.00^{c}	125.56^{cd}	0.9322^{abc}	0.1252^{ab}	0.2929^{ef}	62.19^{ab}	30.73^{c}	55.66^{a}
NSCBR-16	227.44^{a}	93.44^{c}	172.00^{ab}	1.3753^{a}	0.1109^{ab}	0.6746^{b}	68.44^{a}	31.41^{bc}	57.76^{a}
NSCBR-18	147.56^{bc}	113.89^{b}	96.33^{de}	0.7373^{bcd}	0.1681^{ab}	02754^{ef}	72.27^{a}	37.16^{abc}	52.98^{a}
NSCBR-57	204.33^{ab}	80.22^{cd}	135.67^{c}	0.9831^{abc}	0.0878^{b}	0.3430^{de}	68.78^{a}	40.93^{abc}	59.07^{a}
NSCBR-25	213.22^{ab}	93.56^{c}	139.78^{bc}	1.0142^{ab}	0.1103^{ab}	0.4485^{cd}	72.33^{a}	35.38^{abc}	56.44^{a}
–Ve Control	92.44^{cd}	67.56^{de}	85.67^{e}	0.4519^{cd}	0.1035^{ab}	0.2426^{ef}	56.49^{b}	34.76^{abc}	49.11^{a}
+Ve Control	66.28^{d}	55.44^{e}	66.78^{e}	0.2773^{d}	0.2086^{a}	0.1760^{f}	72.66^{a}	37.43^{abc}	53.76^{a}
LSD	72.43	19.70	35.36	0.5404	0.1171	0.1262	11.14	11.41	9.96
Dursitu	219.05^{a}	106.27^{a}	152.37^{a}	1.2439^{a}	0.1935^{a}	0.4688^{a}	61.77^{c}	41.94^{a}	49.14^{c}
Gofta	167.42^{b}	96.77^{b}	120.40^{b}	0.8576^{b}	0.1154^{b}	0.4379^{a}	72.87^{a}	39.85^{a}	55.28^{b}
Kufanzik	131.67^{c}	79.67^{c}	126.03^{b}	0.4955^{c}	0.0766^{b}	0.4594^{a}	67.10^{b}	29.49^{b}	59.80^{a}
LSD	29.24	7.89	14.17	0.2181	0.0469	0.0505	4.49	4.57	3.99
Mean	172.81	94.23	132.93	0.8657	0.1285	0.4553	67.24	37.09	54.74
CV [%]	39.15	13.50	17.17	58.30	58.83	17.89	15.47	19.87	11.75

–VE – negative control (no inoculation and N application), +VE control – 20 kg N ha^{-1}; NSCBR – National soil Common Bean Rhizobium Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

tion whereas N application improved the NDW in soil with a low rhizobial population. In soil with a high rhizobial population, significantly the highest NDW was produced with NSCBR-$(25)_2$, NSCBR-31 and NSCBR-57 inoculation while NSCBR-14 and NSCR-16 inoculation resulted in the highest NDW in soil with a moderate rhizobial population (Figure 1b).

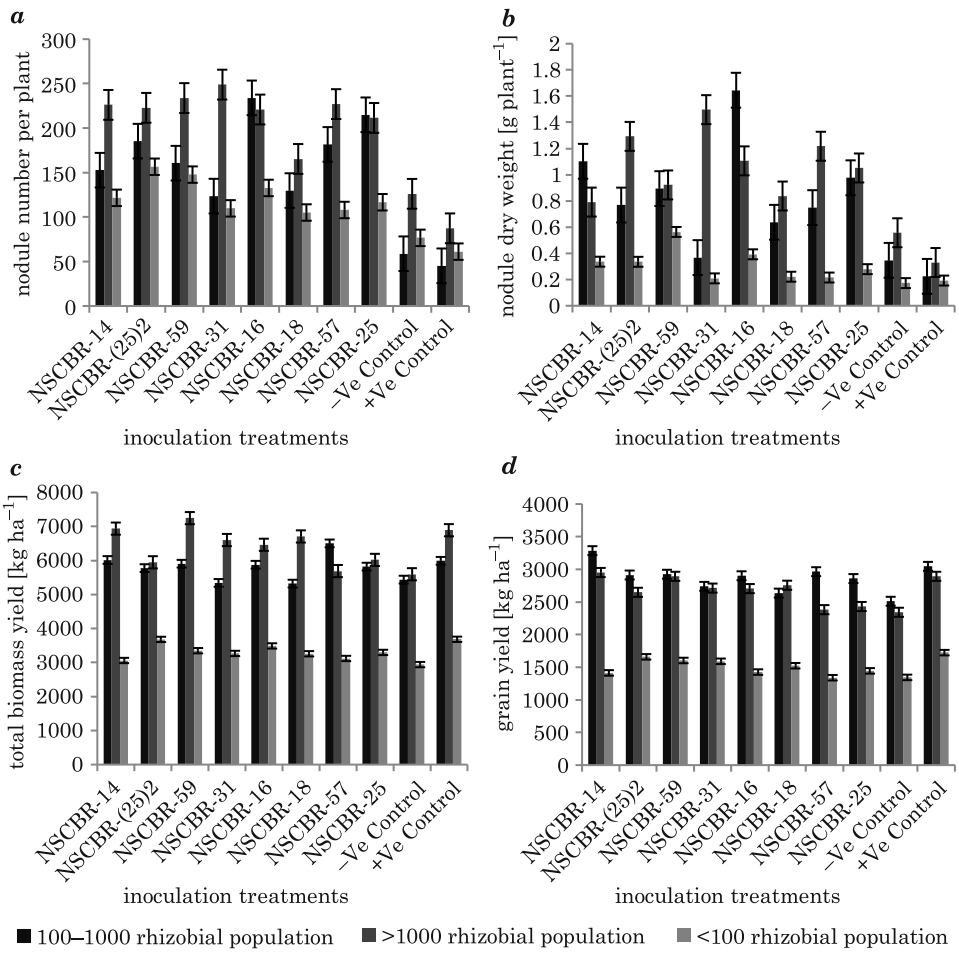

Fig. 1. Effect of elite isolates *Rhizobium* inoculation on: *a* – nodule number, *b* – nodule dry weight, *c* – total biomass yield, *d* – grain yield in soils with different indigenous rhizobial population nodulating common bean

Shoot dry weight

Shoot dry weight (SDW) measured at late flowering and early pod setting stage was significantly ($P < 0.05$) affected by inoculation treatments, the varieties and their interaction except for the main effect of inoculation treatments in soil with a moderate rhizobial population (Table 1). The result revealed the non-significant effect of inoculation on SDW when compared to uninoculated control in soils with a low and moderate rhizobial population (Table 2). In soil containing a high rhizobia population, all isolates except NSCBR-14, NSCBR-$(25)_2$ and NSCBR-31, resulted in significant increase in the SDW.

The highest SDW of common bean in soil with a high rhizobial population was produced at inorganic N application. The non-significant effect of N application on SDW, when compared to the control check in soils with a moderate and high rhizobial population was presented. Among the tested varieties, Gofta produced significantly higher SDW in soil with a high rhizobial population while Dursitu and Gofta gave the better SDW in soil with a low rhizobial population. However, the SDW of Kufanzik was significantly higher than those produced by other varieties in soil containing a moderate rhizobia population.

Number pods per plant

The effect of inoculation treatments, the varieties, and their interaction was significant for a number of pods per plant (NPP) in all soil types except the interaction effect in soil with a high rhizobial population (Table 1). Inoculating NSCBR-$(25)_2$, NSCBR-59 and NSCBR-25 significantly increased NPP in soil with a high rhizobial population (Table 3). In soil containing a low rhizobial population, a significant increase in NPP was noted with NSCBR-57 and NSCBR-25 treatments while this effect was non-significant in soil with a moderate rhizobial population. Though inorganic N application improved the NPP at all soil types, its effect was non-significant when compared to control check. Dursitu variety produced significantly higher NPP than the remaining varieties in all soil types. However, Kufanzik variety produced statistically the same amount of NPP with Dursitu.in soils with a low and moderate rhizobia population.

Table 3

Number of pods per plant, number of seeds per pod and 100 seeds weight as influenced by elite *Rhizobium* isolates inoculation and indigenous *Rhizobium* population nodulating common bean, in eastern Ethiopia, 2013/14

Inoculation	Number of pods per plant			Number of seeds per pod			100 seeds weight [g]		
	> 1000	< 100	100–1000	> 1000	< 100	100–1000	> 1000	< 100	100–1000
NSCBR-14	23.48^{ab}	12.52^{abc}	15.81^{abc}	5.87^{ab}	5.59^{a}	5.59^{a}	28.72^{a}	29.04^{a}	30.83^{ab}
NSCBR-$(25)_2$	23.67^{a}	12.26^{abc}	14.74^{bc}	5.64^{ab}	6.06^{a}	5.52^{ab}	28.53^{a}	27.77^{ab}	31.98^{a}
NSCBR-59	24.70^{a}	11.03^{bc}	16.00^{abc}	5.57^{ab}	5.85^{a}	4.81^{b}	29.44^{a}	27.12^{b}	31.30^{ab}
NSCBR-31	21.96^{abc}	11.25^{bc}	13.93^{c}	5.92^{ab}	5.65^{a}	5.39^{ab}	28.71^{a}	27.82^{ab}	31.03^{ab}
NSCBR-16	23.56^{ab}	10.92^{c}	16.96^{ab}	5.82^{ab}	5.70^{a}	5.00^{ab}	29.84^{a}	27.60^{ab}	32.12^{a}
NSCBR-18	20.17^{bc}	12.66^{abc}	18.40^{a}	5.69^{ab}	5.66^{a}	5.26^{ab}	29.21^{a}	27.00^{b}	31.16^{ab}
NSCBR-57	23.37^{ab}	13.52^{a}	16.00^{abc}	6.14^{a}	5.77^{a}	5.03^{ab}	28.97^{a}	27.12^{b}	31.49^{ab}
NSCBR-25	23.59^{a}	13.44^{a}	17.92^{a}	5.79^{ab}	5.79^{a}	5.01^{ab}	29.23^{a}	27.79^{ab}	30.71^{ab}
–Ve Control	19.20^{bc}	11.04^{bc}	15.59^{abc}	5.47^{b}	5.50^{a}	4.99^{ab}	27.94^{a}	26.89^{b}	30.93^{ab}
+Ve Control	22.71^{ab}	13.00^{ab}	16.37^{abc}	6.01^{b}	5.98^{a}	5.19^{ab}	28.54^{a}	26.96^{b}	30.01^{b}
LSD	3.415	1.97	2.99	0.58	0.99	0.75	2.03	1.48	1.81
Dursitu	24.91^{a}	12.93^{a}	16.68^{a}	6.14^{a}	6.41^{a}	5.28^{a}	19.35^{b}	19.88^{c}	20.90^{b}
Gofta	21.77^{b}	11.31^{b}	15.31^{b}	5.75^{b}	5.64^{b}	5.25^{a}	33.90^{a}	31.98^{a}	36.05^{a}
Kufanzik	21.25^{b}	12.25^{a}	16.53^{a}	5.51^{c}	5.33^{b}	5.01^{a}	33.49^{a}	30.67^{b}	36.53^{a}
LSD	1.38	0.79	1.20	0.233	0.40	0.30	0.82	0.59	0.73
Mean	22.64	12.16	16.17	5.80	5.79	5.18	28.91	27.51	31.16
CV [%]	14.09	10.47	11.94	9.29	11.03	9.30	28.54	3.47	3.76

–VE – negative control (no inoculation and N application), +VE control – 20 kg N ha^{-1}; NSCBR – National soil Common Bean Rhizobium
Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

Table 4

Total biomass yield, grain yield and total plant N accumulation as influenced by elite *Rhizobium* isolates inoculation and indigenous *Rhizobium* population nodulating common bean, in eastern Ethiopia, 2013/14

Inoculation	Total biomass yield [g m^{-2}]			Grain yield [g m^{-2}]			Total plant N accumulation [%]		
	> 1000	< 100	100–1000	> 1000	< 100	100–1000	> 1000	< 100	100–1000
NSCBR-14	647.7^{a}	205.3^{cd}	406.9^{cd}	311.84^{a}	113.11^{c}	169.49^{a-e}	3.3344^{a}	2.4733^{c}	2.7267^{e}
NSCBR-(25)$_2$	586.3^{ab}	243.7^{bc}	492.6^{a}	278.04^{bc}	143.29^{ab}	188.56^{ab}	3.2900^{a}	2.7911^{ab}	3.2567^{ab}
NSCBR-59	657.6^{a}	229.0^{bcd}	440.9^{bc}	290.88^{abc}	144.31^{ab}	176.32^{a-d}	3.2961^{a}	2.3856^{c}	3.0578^{a-d}
NSCBR-31	597.3^{ab}	222.7^{bcd}	431.0^{cd}	272.56^{bc}	125.99^{bc}	192.15^{a}	3.2083^{a}	2.6211^{bc}	3.0778^{a-d}
NSCBR-16	617.0^{ab}	221.0^{bcd}	476.9^{ab}	280.31^{bc}	126.65^{bc}	158.49^{b-e}	3.5206^{a}	2.9967^{a}	3.1289^{abc}
NSCBR-18	601.5^{ab}	208.0^{cd}	443.7^{bc}	269.59^{bcd}	123.29^{bc}	181.26^{abc}	3.1872^{a}	3.0444^{a}	3.2033^{abc}
NSCBR-57	609.7^{ab}	203.7^{d}	420.4^{cd}	267.29^{cd}	124.17^{bc}	143.59^{e}	3.4422^{a}	2.8489^{ab}	3.0356^{bcd}
NSCBR-25	592.0^{ab}	248.1^{b}	411.6^{cd}	264.44^{cd}	143.11^{ab}	145.68^{de}	3.3800^{a}	2.8378^{ab}	3.0111^{cd}
–Ve Control	551.8^{b}	191.3^{d}	396.9^{d}	242.62^{d}	116.75^{c}	152.06^{cde}	3.2339^{a}	2.8956^{ab}	2.8456^{de}
+Ve Control	644.2^{a}	306.1^{a}	430.8^{cd}	296.93^{ab}	163.74^{a}	180.94^{abc}	3.4744^{a}	2.6233^{bc}	3.2911^{a}
LSD	75.7	38.7	37.7	27.83	26.04	32.25	0.5571	0.2881	0.2412
Dursitu	591.6^{a}	177.5^{b}	377.3^{c}	212.28^{c}	100.03^{b}	125.68^{c}	3.8103^{a}	2.8543^{a}	3.5637^{a}
Gofta	621.4^{a}	247.3^{a}	424.5^{b}	289.25^{b}	147.37^{a}	160.40^{b}	3.1040^{b}	2.8210^{a}	2.9283^{b}
Kufanzik	618.6^{a}	258.9^{a}	503.7^{a}	330.84^{a}	149.93^{a}	220.48^{a}	3.0958^{b}	2.5800^{b}	2.6983^{c}
LSD	30.6	15.5	15.1	11.23	10.43	12.92	0.2249	0.1154	0.0966
Mean	610.5	227.9	435.2	277.45	132.44	168.85	3.3367	2.7518	3.0634
CV [%]	11.58	10.95	5.60	9.37	12.70	12.33	15.59	6.76	5.08

–VE – negative control (no inoculation and N application), +VE control – 20 kg N ha^{-1}; NSCBR – National soil Common Bean Rhizobium

Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

Number of seeds per pod

Inoculation, the varieties, and their interaction had significant ($P < 0.05$) effect on the number of seeds per pod (NSP) in soil with a high rhizobial population (Table 1). In soils with a high and moderate rhizobial population, the main effect of the varieties and inoculation treatments was significant on NSP, respectively. Inoculating NSCBR-57 was found to increases significantly the NPP in soil with a high rhizobia population (Table 3). However, inoculation did not improve significantly the NSP in soil with a low and moderate rhizobial population. In general, N application increased the NSP in all soil types, though it was non-significant when compared to that of the control check. Dursitu variety recorded significantly higher NSP than the other varieties in soils with a low and high rhizobia population while non-significant difference among varieties in soil containing a moderate rhizobial population was noted.

One hundred seed weight

ANOVA showed that inoculation, the varieties, and their interaction had a significant effect on 100 seed weight in soils with a low and moderate rhizobia population while only the main effect of the varieties affected significantly the 100-seed weight in soil with a high rhizobial population (Table 1). Inoculating *Rhizobium* did not increase significantly the 100-seed weight in soils with a high and moderate rhizobial population (Table 3). In soil containing a low rhizobial population, a significant increase in 100 seed weight in response to NSCBR-14 inoculation was observed. One hundred seed weight was slightly increased by N application in soils with a high and low rhizobia population. Dursitu and Kufanzik varieties recorded significantly higher 100 seed weight than Gofta variety in soils with a high and moderate rhizobial population while the highest 100 seed weight at soil containing a low rhizobia population was recorded with Gofta.

Total biomass yield

The result of this study showed a significant effect of inoculation, the varieties and their interaction on total biomass yield (TBY) except the interaction effect in soil with a high rhizobia population (Table 1). A significant increase in TBY in response to NSCBR-14 and NSCBR-59 inoculation was recorded in soil containing a high rhizobia population (Table 4). In soil with

a low rhizobia population, NSCBR-$(25)_2$ and NSCBR-25 inoculations resulted in significantly increase in TBY when compared to that of uninoculated control. Inoculating NSCBR-$(25)_2$, NSCBR-59, NSCBR-16 and NSCBR-18were found to significantly superior in TBY in soil with a moderate rhizobial population. All treatments including uninoculated and N fertilized treatments excluding NSCBR-$(25)_2$ and NSCBR-57 resulted in significantly the highest TBY in soil with a high rhizobia population (Figure 1c).

Inorganic N application significantly increased TBY in soils with a high and low rhizobia population. However, the effect of N application on TBY in soil containing a moderate rhizobial population was non-significant when compared to the control check. The TBY among investigated varieties was non-significant in soil containing a high rhizobia population. In soil containing a low rhizobia population had significantly superior TBY with Gofta and Kufanzik while Kufanzik produced the highest TBY in soil with a moderate rhizobial population.

Grain yield

In all soil types, inoculation treatment, the varieties, and their interaction had significant ($P < 0.05$) effect on the grain yield (GY) – Table 1. All isolates excluding NSCBR-18, NSCBR-57, and NSCBR-25, significantly improved the GY in soil containing a high rhizobia population while a significant response of GY to NSCBR-$(25)_2$, NSCBR-57 and NSCBR-25 in soil with a low rhizobia population was noted (Table 4). A significant increase in GY by NSCBR-252 and NSCBR-31 inoculations was observed in soil with a moderate rhizobial population.

Isolates NSCBR-14, NSCBR-$(25)_2$, NSCBR-16, NSCBR-57 and NSCBR-25 inoculation recorded significantly the highest GY in soil containing a high rhizobia population while these isolates recorded significantly the lowest GY in soil with a low rhizobia population (Figure 1d). A significant increase in GY by N application was observed in soils with a high and low rhizobial population. This effect was non-significant in soil with a moderate rhizobial population. Among the investigated varieties, significantly the highest GY was obtained with Kufanzk in all soils types.

Total plant N accumulation

The main effect of inoculation, the varieties, and their interaction had significant ($P <0.05$) on plant N accumulation in soils with a low and moderate

rhizobia population while only the main effect of varieties was significantly affected plant N accumulation in soil containing a high rhizobia population (Table 1). The data revealed the non-significant effect of inoculation on plant N accumulation in soils containing a high and low rhizobia population (Table 4). In soil with a moderate rhizobia population, significantly increase in plant N accumulation in response to NSCBR-$(25)_2$, NSCBR-16 and NSCBR-18 inoculations were noted. Inorganic N application significantly increased the plant N accumulation in soil with a moderate rhizobia population but this effect was not observed in other soil types. In all soil types, Dursitu accumulated significantly the highest plant tissue N over the other varieties.

Discussion

Indigenous rhizobial populations are the major biological factor affecting the effectiveness of inoculated rhizobia and thereby influence the plant productivity (THIES et al. 1991, SCHULZ and THELEN 2008, RUIZ DIAZ et al. 2009, DE BRUIN et al. 2010). However, contradicting findings was reported on the effect of rhizobia population on the effectiveness of *Rhizobium* inoculation on common bean (HUNGRIA et al. 2000, 2003, NDAKIDEMI et al. 2006). Hence, this study was initiated to evaluate the effect of indigenous rhizobial population on the effectiveness of elite isolates of *Rhizobium* inoculation on nodulation, yield and yield traits of common bean grown in eastern Ethiopia. The study sites were categorized into three major groups of soils based on the number of rhizobia nodulation common bean (HOWIESON and BALLARD 2004). Therefore, the soils of Haramaya and Hirna, Fedis soil and Babillae soil were grouped into soils containing a high, moderate and low rhizobia population. Likewise, CHEMINING'WA et al. (2011) observed that indigenous rhizobia are common in central Kenyan soils but varied the number from site to sites. A low rhizobia population at Babillae site could be due to the absence of host legume (GETHI et al. 1997, SÁ 2001) and/or low soil fertility including low SOM and high sand content (CHEMINING'WA and VESSEY 2006, RAPOSEIRAS et al. 2006) and low moisture prevailed in the location (ELIAS and HERRIDGE 2015).

Almost all isolates significantly improved the NN of common bean except NSCBR-18 in soils with a high and moderate rhizobial population while only NSCBR-57 resulted in significantly increase NN in soil containing a low rhizobia population. Similarly, a significant increase in nodule occupation by inoculated isolate in soil with $>10^3$ indigenous rhizobia population nodulating common bean was observed (MRABET et al. 2005, VLASSAK et al. 1996). HUNGRIA et al. (2003) also found that nodules were increased by inoculation of locally isolated *Rhizobium* in soil with a high indigenous rhizobia population.

Nevertheless, isolates which were significantly improved NN did not affect the NDW in all soil types. This indicates that high ineffective isolates could not be effective in increasing nodule biomass. The non-significant effect of inoculation on NDW in Babillae site might be due to a very dry season prevailed in crop growing season and thus reduces the number of naturalized rhizobial (PENA-CABRIALES and ALEXANDER 1979) and suppress the nodule formation (HUNGRIA et al. 2003). The result revealed a significant increase of NDW in soil with a high indigenous rhizobia population. This finding is in line with MULAS et al. (2011) found a significant effect of inoculation on nodulation in soil with a high rhizobial population. HUNGRIA et al. (2003) also observed a significant increase in nodule dry weight of common bean in response to inoculation in soil containing a high rhizobia population.

Shoot dry mass is routinely used to screen varieties for enhanced N_2 fixation (GRAHAM and ROSAS 1977, BUTTERY et al. 1997). This is because large biomass crops require more N, thus N_2 fixation increases as biomass yield increases (HERRIDGE and ROSE 2000). The present study revealed a significant increase in SDW and NSP in response to inoculation in soil containing a high rhizobia population. This result is in accordance with the finding of SILVA et al. (1993) that *Rhizobium* inoculation alone significantly increased shoot biomass up to 57% over uninoculated control. On the other hand, the non-significant effect of inoculation was noted in soils with a low and moderate rhizobia population. This finding might be associated with the dominant effect of environmental and edaphic factors rather than rhizobial population. Beside this, rhizobial competitiveness may have been a major factor affecting the success of inoculation (FERREIRA and HUNGRIA 2002, GRANGE et al. 2007). A significant increase in NPP in response to inoculation was observed in soils with a low and high rhizobia population. However, the effect of inoculation significantly improved 100 seed weight in soil a low rhizobia population.

A significant increase in TBY and GY was observed by inoculated treatments in soil with a high rhizobia population. This confirms the usefulness of *Rhizobium* inoculants for common bean production in Ethiopian cropping system (HUNGRIA et al. 2000). Similarly, HUNGRIA et al. (2003) revealed that strain PRF 81 inoculation increased common bean yield up to178 kg ha^{-1} in soil with a high indigenous population. MOSTASSO et al. (2002) also found statistically similar common bean production between inoculation and N-fertilized control in soil with high indigenous rhizobia population nodulating common bean (10^4 rhizobia per g of soil). This result may be associated with the better competitiveness of inoculated isolates against the background rhizobia nodulating common bean (BEYENE et al. 2004). The indigenous rhizobia nodulating common bean in eastern Ethiopia soils is often relatively ineffective symbionts (AMARE 1988, NSSP 1989, HUBBELL 1995). The positive effect of inoculation in

this soil could be evidenced that N derived from N_2 fixation can support high yield soils poor with N (MENDES et al. 1994, PERES et al. 1994, HUNGRIA et al. 1997, 2000, MOSTASSO et al. 2002). These isolates might produce other growth promoting substance and solubilized insoluble form of nutrients into a soluble form that can easily absorb by plant beside symbiotic N_2 fixation (YADEGARI et al. 2010).

Several studies showed that site-specific yield responses and lack of average response of common bean for *Rhizobium* inoculation are common to inoculant efficiency studies (ABENDROTH et al. 2006, DE BRUIN et al. 2010, FURSETH et al. 2011). In soil with a low rhizobia population, less than three isolates had significantly increased the TBY and GY with the highest values at N fertilized the plant. This indicates that none of the tested isolates fulfill the N requirement of the plant at Babillae site. Failures in inoculation of the common bean could be attributed to lack of competitiveness against indigenous rhizobia due to a harsh environmental condition in the study site (GRAHAM 1981, PEREIRA et al. 1984, BUTTERY et al. 1987, HARDARSON 1993). BERG et al. (1988) found that the competitive ability of different rhizobia strains can influence inoculant efficiency, besides the indigenous rhizobia population size. This finding is contrary to MENDES et al. (1994) that inoculation of common bean varieties increases in grain yield similar to those obtained from 100 kg N ha^{-1} in soil with a low rhizobial population.

Less than four isolates significantly improved the GY and TBY of common bean in soil containing a moderate rhizobial population, indicating that local *Rhizobium* inoculation could be better competent over the indigenous rhizobia even in soil with >100 rhizobial population. PERES et al. (1994) observed that the yield gains due to inoculation of locally isolated rhizobia were ranged from 63 to 290 kg ha^{-1} overuninoculated control. In addition, MULAS et al. (2011) and MRABET et al. (2005) found that some locally isolates *Rhizobium* significantly increased the grain yield common bean in soil with > 1000 rhizobial population. In contrast, these findings, FURSETH et al. (2012) found no yield difference between the inoculated plants and uninoculated check in soil with a high number of rhizobia population. The result of the current study suggests that the rhizobia population level is a poor predictor of common bean response to inoculation in the eastern part of Ethiopia, but environmental and edaphic factors and competitiveness indigenous rhizobia might affect the effectiveness of inoculation.

The result of the present study revealed the non-significant effect of inoculation on plant N accumulation in soils with a low and high rhizobial population. This non-significant effect in soil with a low rhizobial population here could be associated with environmental related limiting factors which might suppress the symbiotic N_2 fixation (TAJINI et al. 2008). In contrast to

this, SILVA et al. (1993) found a significant increase in N_2 fixation by inoculation in soil with a low rhizobial population.

A significant ($P < 0.05$) increase in plant N accumulation in response to three isolates inoculation was observed in soil with a moderate rhizobial population but the highest value was recorded in N fertilized treatment. Similarly, increase in N_2 fixation by *Rhizobium* inoculation of common bean was also reported by HUNGRIA et al. (2000) and MOSTASSO et al. (2002). HUNGRIA et al. (2003) also found a significant increase in plant N concentration up to 465 kg N ha^{-1} by *Rhizobium* inoculation over uninoculated control.

Conclusions

In general, regardless of the indigenous rhizobial population in the soil, some of the tested *Rhizobium* species improved the productivity of common bean. Better nodulation is not the guarantee for good production of common bean. We suggest that the plant growth promoting properties of the isolates may affect the final production of the common bean beside N_2 fixation potential of the isolates. Finally, we recommend that characterizing isolates regarding its plant growth promoting characteristics besides its potential in N_2 fixation would be considered in the selection of *Rhizobium* of common bean for inoculating production. We suggest further research on characterization of competitiveness and effectiveness of indigenous rhizobia beside enumeration of the rhizobial population.

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STARTER FERTILIZATION OF MAIZE AS A METHOD TO IMPROVE THE EFFICIENCY OF NUTRIENT APPLICATION

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K e y w o r d s: *Zea mays* L., nitrogen, phosphorus, method fertilization.

A b s t r a c t

Phosphorus (P) in most regions worldwide is the most important nutrient, second only to nitrogen (N), with the potential to limit agricultural production. It is an essential nutrient for plant growth and development, while the cycle of this nutrient in nature is also essential for humans and animals. In plants it is a component of organic compounds, which accumulate large amounts of energy used in numerous processes taking place in cells. Plants adequately nourished with phosphorus contain more vitamins and carotene, and less oxalic acid, which excess results in deterioration of quality of produced food and feed. At appropriate phosphorus nutrition plants achieve greater efficiency of photosynthesis and are characterized by improved water relations, as a consequence they produce higher grain yields and dry matter yields of the aboveground parts. This study presents original results of five field trails concerning different application methods for nutrients (N and P) in maize culture. Presented data come from controlled field trials, which were conducted at the Department of Agronomy, the Poznań University of Life Sciences.

NAWOŻENIE STARTOWE KUKURYDZY JAKO METODA POPRAWY EFEKTYWNOŚCI APLIKACJI SKŁADNIKA

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A b s t r a k t

Fosfor (P) w większości regionów świata jest najważniejszym pierwiastkiem odżywczym po azocie (N) ograniczającym produkcję rolniczą. Jest niezbędnym składnikiem do wzrostu i rozwoju roślin, a w przyrodniczym obiegu tego składnika jest również konieczny dla człowieka oraz zwierząt. Fosfor spełnia ważne funkcje w procesach życiowych rośliny, takich jak fotosynteza i oddychanie. W roślinie wchodzi w skład związków organicznych, które akumulują dużo energii wykorzystywanej w licznych procesach zachodzących w komórce. Rośliny właściwie odżywione fosforem zawierają więcej witamin i karotenu, a mniej kwasu szczawiowego, którego nadmiar pogarsza jakość wyprodukowanej paszy oraz żywności. Prawidłowe żywienie fosforem powoduje, że rośliny osiągają wyższą wydajność procesu fotosyntezy oraz oszczędniej gospodarują wodą, co w konsekwencji daje zwyżkę plonu ziarna oraz plonu suchej masy części nadziemnej. W opracowaniu omówiono wyniki pięciu badań polowych dotyczących różnych metod aplikacji składników pokarmowych (N i P) w uprawie kukurydzy. Badania te zrealizowano w Katedrze Agronomii Uniwersytetu Przyrodniczego w Poznaniu.

Introduction

Maize (*Zea mays* L.) due to its origin is a thermophyte plants (SOWIŃSKI 2000). For adequate growth and rapid development it needs more heat in the vegetation period than other cereals. The effect of temperature is manifested e.g. in the dynamics of dry matter accumulation and initial growth rate (KRUCZEK and SZULC 2006). Low soil and air temperatures at sowing and in the initial phases of growth in maize are primary causes reducing its yielding. Additionally, in the spring cold spells occurring at various development stages of maize retard its growth. In Poland, where maize is sown in the third decade of April and in the first days of May we frequently have to deal with adverse temperature conditions. Maintenance of advantageous nutrition conditions facilitating acceleration of growth rate, particularly in the initial phases of its development, has a positive effect on yielding (SZULC 2013).

Slow initial growth caused by too low temperatures during the vegetation period of maize, as it was shown in recent studies, is a result of reduced uptake of water and nutrients, particularly phosphorus (MOZAFAR et al. 1993). Moreover, in that time the underdeveloped radicle system may supply plants with minerals only at their adequately high concentration in the soil medium. In turn, an adequate concentration of phosphorus in the soil medium is required for a rapid development of the root system in maize and it facilitates alleviation of effects of nutritional stress (YANAI et al. 1996). The concentration of phosphates in comparison to other anions is very low, as the share of the phosphate ion in the total anions in the soil medium is maximum several percent (MOLLIER and PELLERIN 1999).

These problems may be overcome applying various cultivation measures, fertilizer types, particularly mixed fertilizers, as well as their application site (SZULC et al. 2016a, SZULC et al. 2016b) One of the methods to enhance

availability of phosphorus in the immediate vicinity of roots is broadcasting large doses of phosphorus fertilizers. In order to ensure uptake of this nutrient by plants under adverse conditions these doses have to considerably exceed nutrient requirements of maize. Such an approach is neither economically viable nor acceptable from the point of view of environmental protection (SHARPLEY et al. 2001, LADHA et al. 2005, SZULC 2010, SZULC et al. 2015). Excessive phosphorus contents in the soil lead to losses of the nutrient due to surface runoff, increasing eutrophication. Agriculture is not a closed system and some of the accumulated phosphorus is released to the environment. Standard broadcasting fertilization does not always ensure adequate plant nutrition, since depending on soil properties some of the nutrient introduced to the soil in the form of fertilizer will be retrograded, particularly in soils with considerable potential for immobilization, or it will be deposited in sites outside the reach of roots of crops (EL-HAMDI and WOODARD 1995**).**

A much better method to enhance phosphorus availability is to place fertilizer in the immediate vicinity of seeds. Such a method of fertilizer application is referred to as row, starter or topical. It consists in the placement of fertilizer 5 cm deeper and 5 cm away from seeds **(**MURPHY 1984, MASCAGNI and BOQUET 1996, RHOADS and WRIGHT 1998**)**. It causes better supply of young plants with nutrients, it accelerates their vegetation, while additionally it affects grain yield. Starter fertilization also makes it possible to reduce the dose of phosphorus thanks to its better utilization in the year of application and it reduces the rate of its retrogradation in soils with low resources of this nutrient. Moreover, such a method of phosphorus application results in the deposition of this nutrient in a deeper, moister soil layer, ensuring its improved uptake. This is particularly important in the case of nutrients of limited mobility, such as phosphorus (KRUCZEK and SZULC 2006).

The first studies in Poland concerning the effect of row (starter) application of phosphorus and mixed fertilizers on the course of vegetation and yielding of maize were conducted at the Poznan University of Life Sciences (formerly the Agricultural University) in the 1970's (DUBAS and DUHR 1983**)**. These studies confirmed the assumption that when soil temperature at a depth of 5 cm (sowing depth) at sowing up to the development of 3–4 leaves did not exceed 5.5°C starter fertilization not only increased phosphorus uptake by plants, but also significantly affected grain yield in relation to conventional broadcasting (DUBAS and DUHR 1983**)**. Thus considering frequent long-term spring cold spells in Poland during sowing and the initial period of maize growth it may be assumed that fertilization performed jointly with sowing as starter fertilization will be particularly efficient. It seemed that advisability of starter fertilization in Poland should thus be confirmed by research results. This was also connected with the dramatic change in the selected maize cultivars, in

which breeding progress altered their environmental requirements, especially temperature conditions. Moreover, changes were also introduced to offered phosphorus and mixed fertilizers, exhibiting improved availability. For this reason at the Department of Agronomy (formerly the Department of Soil and Plant Cultivation) studies were initiated on the advisability of such a method of fertilizer application in maize growing. This study presents results of five field trials on such a fertilization method. In view of the extensive scope of the research problem only three traits were focused on: (i) the dynamics of initial growth, (ii) grain yield, and (iii) grain moisture content. Moreover, the paper presents and discusses factors determining effectiveness of various maize fertilization methods as well as mechanisms of nutrient uptake by plants.

Thermal and humidity condition in the years of conducting field tests in which the described research tasks were carried out

Air temperature (Table 1) in April and early May, during sowing and emergence of plants, except for 2000 and 2007, was unfavorable for maize as it was less than 10°C. In the later growing seasons, in the maize-critical flowering and flushing (the second half of June and early July), the weather conditions were favorable for most years. Only in the years 2000 and 2001 were they below the requirements of maize. In August and September, thermal conditions, in spite of their differentiation in particular years, generally favored the formation of grains and its maturation. The humidity conditions (Table 2) in the experimental years were more varied than the air temperature. They express not only differences in the sum of precipitation in the vegetation

Table 1

Average month air temperatures [°C] in growing seasons

Years	Months							
	IV	V	VI	VII	VIII	IX	X	Average for IV–X
2000	12.1	15.7	17.5	16.3	18.5	12.9	12.1	15.0
2001	8.3	15.2	15.3	19.9	19.3	12.2	12.3	14.6
2002	8.9	16.8	18.1	20.6	21.4	14.1	7.3	15.3
2003	8.6	15.7	19.2	19.8	20.0	15.1	5.7	14.8
2004	9.7	12.9	16.1	18.2	20.1	14.2	10.4	14.5
2005	9.4	13.3	16.5	19.9	17.3	16.0	10.5	14.7
2006	8.8	13.8	18.7	24.4	17.7	17.2	11.3	15.9
2007	10.8	15.2	19.3	18.9	19.2	13.7	8.5	15.1

Table 2

Month rainfalls [mm] in growing seasons

Years	Months							
	IV	V	VI	VII	VIII	IX	X	Sum for IV–X
2000	15.7	47.4	29.9	73.0	95.6	38.8	11.8	312.2
2001	33.1	10.4	67.8	65.8	44.6	119.3	31.9	372.9
2002	34.2	45.7	38.1	29.6	56.1	15.8	89.3	308.8
2003	16.2	24.0	40.4	97.7	5.8	15.9	31.6	231.6
2004	19.4	49.8	51.3	49.4	53.6	32.3	45.2	301.0
2005	14.5	74.3	19.1	97.4	60.7	34.4	5.0	305.4
2006	43.6	57.4	26.9	23.1	100.7	22.0	22.1	295.8
2007	9.3	77.0	59.6	87.0	48.1	33.4	18.5	332.9

periods, but especially their distribution in particular months. The monthly precipitation totals ranged from 231.6 mm in 2003 to 372.9 mm in 2001. Their distribution was very different at this time and was characterized by violent precipitation or cumulative short-term and unsuitable water retention in soil or longer periods without precipitation causing periodic water shortages in the soil.

Factors determining effectiveness of different nutrient application methods

Mechanism of phosphorus uptake

Soil provides water and nutrients and they are absorbed by roots (GRZEBISZ 1988). Nutrient uptake, including phosphorus, through roots is dependent on appropriate plant turgor. Plants adequately supplied with water absorb greater amounts of this nutrient transferring it to zones of intensive growth. Ions from the soil medium are absorbed thanks to three basic mechanisms: by root contact with the ion, through movement of ions with water and through diffusion (GRZEBISZ and GAŁA 1999).

The first of the three processes, in which the nutrient penetrates to plant roots consists in the direct contact of soil with the roots. It is responsible to a slight extent for the amount of nutrients, which may reach root surface. In this way approx. 6% nutrient requirements of plants may be met (GRZEBISZ 1990). In the immediate area of the roots soil phosphorus resources are quickly depleted, causing a decrease in the concentration of this nutrient.

In order to provide adequate plant phosphorus nutrition it needs to be supplied from more distant soil zones. From these zones ions may penetrate

towards the roots flowing with water or as a result of diffusion. The amount of nutrients reaching the roots in this way is dependent on the water flow rate and on its uptake by plants, as well as the concentration of a given nutrient in the soil medium. However, irrespective of soil fertility, mass flow does not always satisfy nutrient requirements of plants in relation to phosphorus. As it was reported by CAMPBELL et al. (1989) the limited importance of water flow may be explained by the low concentration of phosphorus in the soil medium. As a result of water flow plants may absorb as little as 1–10% required amounts of phosphorus. Mechanisms of ion transport in the soil towards the physiologically active root surface in the case of phosphorus a decisive role is played by the process of diffusion. Diffusion consists in the flow of ions in accordance with the gradient of concentration caused by the presence of plant roots. Plants, by absorbing the ion, cause its movement towards the roots, i.e. absorb it according to the gradient of concentration. This process occurs in the direction towards plant roots, when ion concentrations in this zone are reduced or in the opposite direction when concentrations of ions in the root zone are greater than in the soil medium. The rate of ion movement is dependent on the values of ion diffusion coefficients and on soil moisture content. Movement of ions, irrespective of the mechanism of this process, always takes place in water. The dependence of diffusion rate on soil moisture content may be explained by the increase in the share of pores filled with air with the decrease in soil moisture content. The moister the soil is, the faster (shorter) the path of phosphorus to plant roots. As it was reported by BHADORIA et al. (1991), at soil saturation with water the rate of ion diffusion towards the roots is dependent on the difference in concentrations on the root surface and in the soil. Considering weak solubility of phosphorus and its low concentration in the soil solution the amount of the active nutrient in the soil is determined by soil moisture content. Phosphorus in the soil solution is supplemented from the soil solid phase, on condition a sufficient amount of water is present. In soils of low water holding capacity or during drought the concentration of phosphorus in the soil medium should be greater for plants to be able to absorb the amount of this nutrient required for their development. In practice this means that greater doses of fertilizers have to be applied. As it was reported by ŁABĘTOWICZ and RUTKOWSKA (2001) concentration of phosphates in the soil medium is crucial for the uptake of this macronutrient by crops.

The effect of temperature on phosphorus uptake

Studies conducted by many authors indicate that changing soil temperature has a tremendous effect on many mechanisms involved in nutrient uptake

by plants (MURPHY 1984). A decrease in soil temperature (below 10–12°C) reduces the rate of organic matter mineralization, solubility of inorganic forms of phosphorus, permeability of cytoplasmic membranes, while it also limits root activity (KRUCZEK 2005e). A decrease in soil temperature leads to a reduced uptake of several essential ions, particularly phosphorus. A low soil temperature increases viscosity of the soil solution and reduces the rate of diffusion, thus decreasing the amount of absorbable phosphorus, which reaches root surface. An increase in temperature by 1°C causes an increase in phosphorus contents in the soil solution by 1–2%. One of the functions of roots is connected with nutrient uptake from the substrate. Maize plants due to their abundant growth have a very well developed root system, which supplies them with adequate amounts of water and nutrients. As it was reported by SOWIŃSKI and MALISZEWSKI (1989), root pressure responsible for shoot supply with minerals is reduced at a low temperature. In turn, phosphorus is the only ion, which uptake is dependent particularly on root activity.

Experiments conducted by MACKAY and BARBER (1984) at a soil temperature of 25°C the total yield of maize (roots and shoots) was by 4–6.5-fold greater than at 18°C, root growth was by 2.6 to 5.1 times greater, while phosphorus uptake was by 2- to 4-fold greater. An increase in air temperature to 25°C at soil temperature of 18°C caused a 2.7-fold greater growth of roots and 2.2-fold greater phosphorus uptake. CHING and BARBER (1979) showed that growth rate of maize roots at 15°C was by 50% lower than at 29°C. DIBB et al. (1989) also stated that an increase in soil temperature from 5°C to 27°C caused an increase in growth of aboveground parts in maize by approx. 400%, while uptake of phosphorus by 275%. MOZAFAR et al. (1993) investigated the effect of day length and soil temperature in the root zone on growth and contents of nutrients as well as their distribution in maize. A 3-fold increase in day length from 6 to 18 h had no effect on growth of aboveground parts of plants and roots if soil temperature in the root zone was 9°C, but it increased each of the parameters 8-fold if the temperature of the root zone was 21°C. Interacting day length and the temperature of the root zone showed a completely different effect on contents of nutrients in both roots and aboveground parts of plants. An extension of day length at a given temperature of the root zone reduced nutrient concentrations, while an increase in soil temperature in the root zone in a given lighting period increased contents of most elements both in roots and the aboveground parts. A lack of response in maize to an extended day length, manifested in plant growth on condition that roots are subjected to low soil temperatures, indicates a dominant role of temperature in the root zone over the role of day length at early development phases of maize.

Response of cultivars to starter fertilization

An important factor determining the effectiveness of topical fertilization is connected with an appropriate selection of cultivars. It results from studies conducted by MASCAGNI and BOQUET (1996) and TEARE and WRIGHT (1990) that not all cultivars respond positively to starter fertilization in terms of the volume of grain yield. There are cultivars responding to starter fertilization consistently with an increased grain yield, cultivars consistently responding negatively and hybrids indifferent to this method of fertilizer application. Cultivars, which are highly sensitive to temperature, are more prone to respond to starter application of phosphorus when the temperature is below normal (RHOADS and WRIGHT 1998). Moreover, those authors ascribed the response of cultivars to fertilizer application methods (broadcasting, row application) on weather conditions in the years of the study. They reached a similar conclusion as the previously cited authors, stating that certain cultivars respond with increased grain yields irrespective of weather conditions over the years, while the response of the other cultivars varies over the years. Cultivars with a high growth rate of roots and high uptake rates of N and P exhibit a lesser response to the starter fertilizer. As it was reported by RHOADS and WRIGHT (1998), a positive constant response of maize to topical fertilization is observed in the case of low soil temperatures, which limit growth rate of roots. Thus it results from conducted analyses that the hybrids with a low root growth rate and a low nutrient uptake rate react positively to starter fertilization.

Selection of fertilizer for starter fertilization

Optimal choices for starter fertilization include two-component fertilizers containing nitrogen and phosphorus. Changes of phosphorus compounds in the soil are dependent on the presence of accompanying salts, of which the greatest effect is found for nitrogen compounds (MURPHY 1984). Fertilizer pellets after being introduced to the soil dissolve very rapidly. In the vicinity of pellets a saturated phosphate solution is formed in relation to the most readily soluble compounds contained in the fertilizer. The composition and concentration of this solution are not dependent on soil properties, but solely on fertilizer properties. Ions from the concentrated solution diffuse outside fertilizer pellets, while water flows in the opposite direction. With an increase in the distance from the fertilizer pellet phosphates undergo chemical and physico-chemical sorption, causing a decrease in the concentration of phosphorus in the soil solution. A combination of two components, N and P, increases the

uptake of phosphorus by maize during initial development phases (KRUCZEK 2005e). However, it needs to be remembered that the rate of phosphorus absorption depends on the form, in which plants absorb nitrogen. At plant nutrition with ammonia nitrogen $N\text{-}NH_4$ H^+ is released from the cells to the soil solution, causing its acidification, which as a rule increases the concentration of phosphorus and the rate of its absorption. At plant nutrition with the nitrate form $N\text{-}NO_3$ ions of HCO_3^- and OH^- are secreted from cells, causing alkalization of the soil solution, at the same time reducing absorption of phosphorus. At phosphorus deficit in the plant environment plants absorb little nitrogen, while the uptake of nitrogen is limited also at an excessive dose of phosphorus. For this reason only an N : P ratio adequate for a given plant ensures appropriate growth and development. It results from pot experiments conducted by SEIDLER and GÓRSKI (1986) that the greatest plant height and total leaf blade area in maize plants were obtained at a nitrogen to phosphorus ratio of 1:0.6. In turn, SEIDLER and GÓRSKI (1984, 1980) in similar experiments showed that the application of phosphorus in the nutrient medium in the amounts close to the nitrogen dose of 1:0.8 provided the best values of analyzed parameters in maize (net assimilation rate NAR, yield of dry matter). These authors explained it by a greater efficiency of photosynthesis, as well as more efficient water management of plants. Doses of phosphorus greater than those of nitrogen at 1:1.6 disturbed physiological processes, resulting in dry matter yield depression.

Results of studies on maize fertilization methods

Experiment 1. The effect of method of fertilization with phosphorus and mixed fertilizer on growth and development as well as yielding in maize

The effect of starter fertilization on the rate of dry matter accumulation by maize in the initial period of development was manifested starting from the phase of 4–5 leaves and it increased with progress in vegetation (Table 3). Dry matter of the aboveground parts of a single plant as a result of row fertilization in comparison to broadcasting was by 10.8% higher at the phase of 4–5 leaves, by 41.8% at the phase of 6–7 leaves and by 60.9% at the phase of 8–9 leaves. A greater dry matter of the aboveground parts of a single plant under the influence of row fertilization was obtained not only irrespective of weather conditions, but also in all the 4 years of the study (Table 3). Those years in the initial development phases of maize were characterized by moisture deficit in the soil, showing that thanks to starter fertilization the action of fertilizer is

independent of periodical water deficits in the spring as a consequence of a more advantageous deposition of fertilizer in a deeper soil layer, moister thanks to the upward water movement. Thus these experiments did not confirm a conjecture of a greater effectiveness of starter fertilization at low temperatures found in spring. It resulted from advantageous temperature conditions in all the four years of the study. Row application of a single-component fertilizer (triple superphosphate) and two-component fertilizer (ammonia phosphate) had an advantageous effect on the accumulation of dry matter by maize at initial growth phases. A greater effectiveness was recorded in the case of ammonia phosphate (Figure 1). Row application of both triple

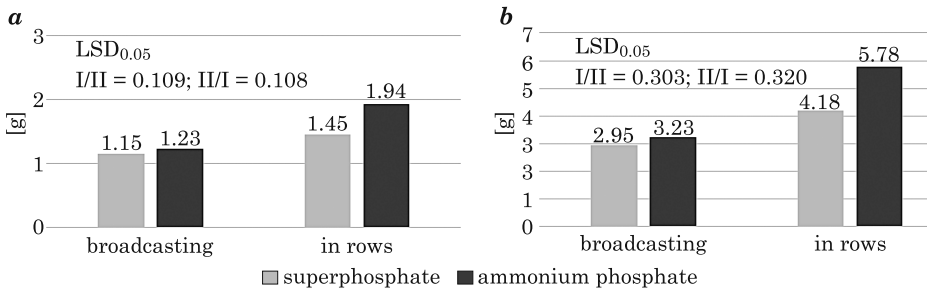

Fig. 1. Dry mass of above-ground part of one plant in phases of 6–7 leaves (a) and 8–9 (*b*) in dependence on kind of fertilizer and method of fertilization (2000–2003) (KRUCZEK and SZULC 2005a)

superphosphate and ammonia phosphate had an advantageous effect on the accumulation of dry matter by maize at initial growth phases. However, a markedly greater effectiveness was recorded in the case of ammonia phosphate. Dry matter of the aboveground parts increased with an increase in phosphorus fertilization ranging from 40 to 130 kg P_2O^{35} ha^{-1} at the phase of 8–9 leaves (Table 3). Row application of fertilizer significantly increased grain yield in maize in relation to row application, and it was irrespective of weather conditions in the years of the study, type of fertilizer and soil phosphorus resources (Table 4). Moisture content in the period of flowering and grain setting by maize were factors determining the effectiveness of row fertilization. Optimal soil moisture content in that period provided the greatest increase in grain yield as a result of row fertilization, irrespective of precipitation total throughout the vegetation period. Starter broadcasting of ammonia phosphate significantly increased grain yield in relation to row application. In the case of triple superphosphate the method of fertilization did not change grain yields (Figure 2).

Irrespective of weather conditions, water content in grain depended solely on the method of fertilizer application (Table 5). A significantly lower moisture

content was recorded in treatments, in which fertilizer was applied in rows (27.5%), in comparison to treatments with conventional row application (27.9%). This dependence was observed in all the years of the study, while it was confirmed statistically in the years 2000 and 2003.

Table 3

Dry mass of above-ground parts of 1 plant [g] (2000–2003) (KRUCZEK and SZULC 2005a)

Specification		Sampling dates				
		full emergency	2–3 leaves	4–5 leaves	6–7 leaves	8–9 leaves
Average for control		0.05	0.08	0.29	1.04	2.47
P_2O_5 dose [kg ha^{-1}]	40	0.05	0.09	0.32	1.43	3.65
	70	0.05	0.08	0.32	1.40	4.04
	100	0.05	0.08	0.32	1.48	3.86
	130	0.05	0.08	0.35	1.46	4.58
$LSD_{0.05}$		n.s.	n.s.	n.s.	n.s.	0.390
Kind of fertilizer	superphosphate	0.05	0.08	0.31	1.30	3.57
	ammonium phosphate	0.05	0.09	0.34	1.59	4.50
$LSD_{0.05}$		n.s.	0.002	0.013	0.076	0.238
Fertilization method	broadcast	0.05	0.08	0.31	1.19	3.09
	in rows	0.05	0.08	0.34	1.69	4.98
$LSD_{0.05}$		n.s.	n.s.	0.010	0.072	0.215

n.s. – no significant differences

Table 4

Yield of maize grain [dt ha^{-1}] (KRUCZEK and SZULC 2005b)

Specification		Years				Average
		2000	2001	2002	2003	
Average for control		92.5	91.2	59.0	104.6	
P_2O_5 dose [kg ha^{-1}]	40	94.2	94.7	62.4	103.3	88.7
	70	97.6	93.7	60.0	105.4	89.2
	100	84.6	95.6	55.9	107.8	86.0
	130	94.5	94.4	59.0	109.2	89.3
$LSD_{0.05}$		8.46	n.s.	n.s.	1.41	n.s.
Kind of fertilizer	superphosphate	93.0	94.3	58.7	106.0	88.0
	ammonium phosphate	92.5	94.9	60.0	106.8	88.5
$LSD_{0.05}$		n.s.	n.s.	n.s.	n.s.	n.s.
Fertilization method	broadcast	91.5	94.5	58.6	105.7	87.6
	in rows	93.9	94.7	60.1	107.1	89.0
$LSD_{0.05}$		1.76	n.s.	n.s.	1.21	0.95

n.s. – no significant differences

Table 5

Grain moisture [%] (KRUCZEK and SZULC 2005b)

Fertilization method	Years				Average
	2000	2001	2002	2003	
Broadcast	33.0	27.3	28.5	22.6	27.9
In rows	32.3	27.0	28.1	22.4	27.5
$LSD_{0.05}$	0.49	n.s.	n.s.	0.18	0.19

n.s. – no significant differences

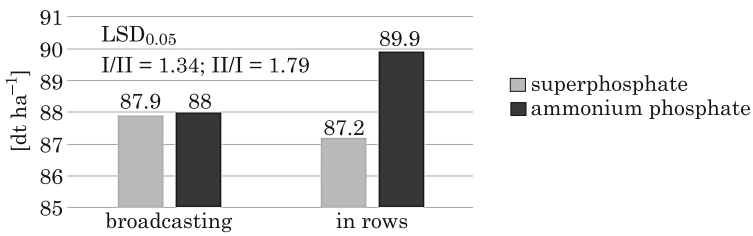

Fig. 2. Effect of fertilization method and kind of fertilizer on grain yield (2000–2003) (KRUCZEK and SZULC 2005b)

Experiment 2. The effect of method of fertilization with nitrogen and mixed fertilizer on development and yielding of maize

Topical fertilization, applied jointly with grain sowing, had an advantageous effect on the initial growth of maize plants, irrespective of weather conditions. It was manifested in an increased dry matter of a single plant at the phase of 4–5 leaves, in comparison to broadcasting (Table 6). A positive effect of row fertilization on these traits was particularly strong at low doses of nitrogen, while at doses above 95 kg N ha^{-1} this effect was not observed (Figure 3). The greatest positive effect of starter fertilization on increment in dry matter of a single plant in comparison to broadcasting was obtained using a mixed fertilizer, hydrofoska, while it was lower following the application of ammonium nitrate and the lowest after urea application. Starter broadcasting of nitrogen fertilizers or mixed fertilizer gave better effect in grain yield than their broadcasting, irrespective of weather conditions, the level of nitrogen fertilization and the type of fertilizer (Table 7). Starter fertilization significantly increased grain yield in comparison to conventional fertilization (broadcasting) in the years, in which precipitation total in the vegetation period was comparable to the multiannual mean. The type of nitrogen fertilizer (urea and ammonium nitrate) or mixed fertilizer (hydrofoska 21) did not cause changes in grain yield of maize, either at row or broadcasting application, and it was irrespective of the method of their application (Table 7).

Table 6

The dry mass of above-ground parts of 1 plant in phase 4–5 leaves [g] (KRUCZEK 2004a)

Specification		Years				Average
		2000	2001	2002	2003	
N dose [kg ha^{-1}]	25	0.48	0.31	1.31	1.61	0.93
	60	0.50	0.33	1.36	1.53	0.93
	95	0.47	0.34	1.23	1.38	0.85
	130	0.48	0.34	1.32	1.36	0.87
$LSD_{0.05}$		n.s.	n.s.	n.s.	n.s.	n.s.
Kind of fertilizer	urea	0.46	0.33	1.31	1.49	0.90
	ammonium nitrate	0.49	0.33	1.28	1.36	0.87
	hydrofoska	0.50	0.32	1.32	1.56	0.92
$LSD_{0.05}$		0.022	n.s.	n.s.	0.110	0.039
Fertilization method	broadcast	0.47	0.32	1.26	1.36	0.85
	in rows	0.50	0.34	1.35	1.58	0.94
$LSD_{0.05}$		0.02	0.01	0.04	0.07	0.02

n.s. – no significant differences

Table 7

Yield of the maize grain [dt ha^{-1}] (KRUCZEK 2005a)

Specification		Years				Average
		2000	2001	2002	2003	
Average for control		82.5	65.7	59.3	67.0	
N dose [kg ha^{-1}]	25	92.4	81.7	61.0	78.5	78.4
	60	101.0	94.5	65.1	90.6	87.8
	95	103.7	102.6	58.8	98.9	91.0
	130	107.4	96.6	64.0	90.0	89.5
$LSD_{0.05}$		5.8	8.9	n.s.	n.s.	5.4
Kind of fertilizer	urea	12.2	93.6	64.2	85.4	86.4
	ammonium nitrate	100.0	93.2	60.6	91.9	86.4
	hydrofoska	101.1	94.7	61.8	91.3	87.2
$LSD_{0.05}$		n.s.	n.s.	n.s.	n.s.	n.s.
Fertilization method	broadcast	100.0	92.9	61.2	89.2	85.9
	in rows	102.2	94.8	63.2	89.8	87.5
$LSD_{0.05}$		1.9	1.4	n.s.	n.s.	1.0

n.s. – no significant differences

The method of fertilizer application did not change water content in grain at the application of nitrogen doses of 25, 60 or 95 kg N ha^{-1}. In turn, row application of 130 kg N ha^{-1} significantly increased moisture content in grain in comparison to broadcasting. Doses of nitrogen did not alter moisture

content in grain during harvest if row fertilization was applied together with sowing. In the case of fertilizer broadcasting the moisture content in grain from treatments with the fertilizer dose of 95 kg N ha^{-1} was significantly greater than in treatments with doses of 60 and 130 kg N ha^{-1}, in the case of which water content in grain was comparable (Figure 4).

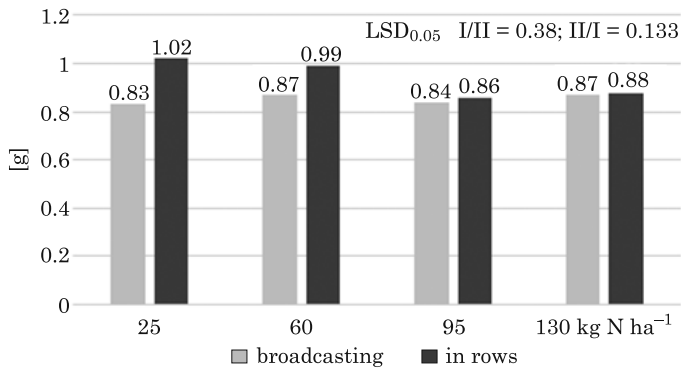

Fig. 3. The dry mass of above-ground parts of one plant (2000–2003) (KRUCZEK 2004a)

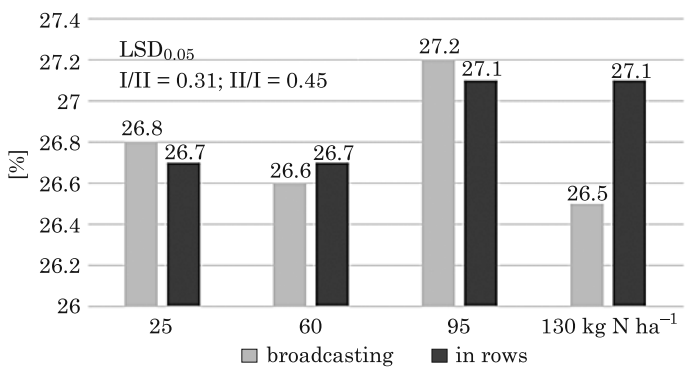

Fig. 4. Effect of nitrogen rate and fertilization method on moisture of grain (2000–2003) (KRUCZEK 2005a)

Experiment 3. Response of maize cultivars to the date and method of fertilization with two-component fertilizer (NP)

In studies concerning the response of hybrid maize cultivars to the method of fertilizer application at different sowing dates it was shown that starter broadcasting of ammonia phosphate significantly increased initial growth rate of maize, which was manifested in the production of dry matter of aboveground

Table 8

The dry mass of above-ground parts of 1 plant in phase 4–5 leaves [g] (KRUCZEK 2004b)

Specification		Years				Average
		2000	2001	2002	2003	
Varieties	Janna	0.82	0.21	1.21	0.84	0.77
	Costella	0.85	0.34	0.99	0.72	0.73
	Marignian	1.09	0.35	1.52	0.99	0.99
$LSD_{0.05}$		0.11	0.03	n.s.	0.20	0.12
Sowing date	12 IV	0.31	0.26	0.58	0.72	0.46
	26 IV	0.75	0.34	1.61	0.96	0.92
	10 V	1.71	0.30	1.53	0.88	1.11
$LSD_{0.05}$		0.09	0.02	0.24	0.10	0.07
Fertilization method	broadcast	0.84	0.27	1.04	0.71	0.72
	in rows	1.01	0.33	1.43	0.99	0.94
$LSD_{0.05}$		0.05	0.01	0.12	0.08	0.05

n.s. – no significant differences

Table 9

Yield of the maize grain [dt ha^{-1}] (KRUCZEK 2005b)

Specification		Years				Average
		2000	2001	2002	2003	
Varieties	Janna	83.7	76.2	51.9	83.9	73.9
	Costella	108.4	96.4	75.0	99.0	94.7
	Marignian	111.3	96.6	63.5	94.7	91.5
$LSD_{0.05}$		8.0	7.0	n.s.	0.8	5.2
Sowing date	12 IV	100.2	86.0	63.0	97.6	86.7
	26 IV	101.6	90.8	64.1	92.6	87.3
	10 V	101.6	92.3	63.3	87.4	86.2
$LSD_{0.05}$		n.s.	2.8	n.s.	1.2	n.s.
Fertilization method	broadcast	99.7	88.3	63.4	90.2	85.4
	in rows	102.6	91.1	63.6	94.9	88.0
$LSD_{0.05}$		1.6	2.2	n.s.	0.9	0.9

n.s. – no significant differences

parts of 1 plant at the phase of 4-5 leaves by 30.9% greater than in the case of broadcasting (Table 8). Row fertilizer application increased dry matter of 1 plant at this development phase at all sowing dates. Dry matter of aboveground parts of 1 plant at the phase of 4–5 leaves increased with a delay in sowing date from 12 April to 10 May (Table 8). This increase was more marked in treatments with starter fertilization in comparison to treatments with broadcasting fertilization. Irrespective of weather conditions and soil

phosphorus resources, starter application of ammonia phosphate contributed to an increase in grain yield of maize in comparison to broadcasting (Table 9). Starter fertilization in maize using two-component fertilizer NP was far more effective than broadcasting not only at the optimal sowing date (26 April), but also at dates accelerated or delayed by 2 weeks (Figure 5). No varied response of maize cultivars to fertilization method was observed. Cultivars Janna, Costella and Marignian differed in their response to sowing date. The most advantageous sowing date for yielding of the earliest cv. Janna was 26 April, while for the latest-yielding cultivar Marignian it was 12 April. The date of sowing had no effect on yielding in medium early cv. Costella. Among the investigated factors moisture content of grain was influenced by cultivar and the date of sowing. As it could have been expected, the lowest moisture content at harvest was recorded in grain of the earliest cv. Janna, followed by the later cv. Costella, while it was greatest for the latest cv. Marignian. Differences in moisture content in grain between all the tested cultivars were statistically significant. On average for the 4 years of the study a delay in sowing date from 12 April to 10 May gradually increased grain water content. In comparison to the optimal sowing date of maize, i.e. 26 April, acceleration of sowing by 2 weeks reduced moisture content in grain by 0.9%, while delay in sowing by 2 weeks increased grain moisture content by 3.8%.

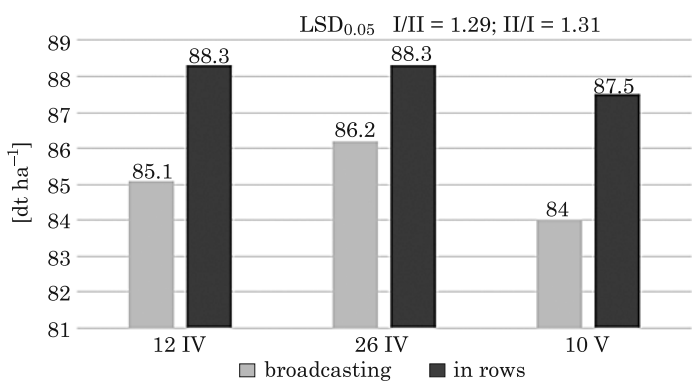

Fig. 5. Effect of sowing date and fertilizing method on the grain yield (2000–2003) (KRUCZEK 2005b)

Experiment 4. The effect of fertilization method for different types of fertilizers on growth development and yielding of maize

Studies concerning the method of fertilization using different types of fertilizers showed the most advantageous effect on yield of dry matter in the initial period of maize growth for row application of fertilizers. in which

P content was lower than that of N or it slightly exceeded it as in the case of hydrofoska 16 (N:P = 1:0.44). polifoska 8 (N:P = 1:1.31) and ammonia phosphate (N:P = 1:1.11). Row application of amofoska NPK/S. i.e. a fertilizer. in which P content markedly exceeded N content (N:P = 1:1.74). did not stimulate initial growth in maize plants (Table 10). Row application of fertilizers had a positive effect on grain yield in maize in comparison to broadcasting. particularly in the years. in which the distribution of precipitation promoted high yields. In the year characterized by rainfall deficit throughout the vegetation period no such effect was observed (Table 11).

Table 10

The yield of dry mass of above-ground parts of in the phase of 5–6 leaves [kg ha^{-1}] (KRUCZEK 2005c)

Studied factors	Years			Mean
	2001	2002	2003	
Hydrofoska 16; N:P = 1:0.44	32.9	40.0	103.8	58.9
Amofoska N:P = 1:1.74	28.9	36.6	54.1	39.9
Polifoska 8; N:P = 1:1.31	36.1	49.2	108.3	64.5
Ammonium phosphate N:P = 1:1.11	34.0	49.8	113.0	65.6
Superphosphate	33.3	40.2	74.8	49.4
$LSD_{0.05}$	6.62	9.62	28.80	9.49
Broadcast fertilization	31.2	41.8	56.0	43.0
In rows fertilization	34.9	44.6	125.6	68.3
$LSD_{0.05}$	2.1	n.s.	14.4	4.8

n.s. – no significant differences

Table 11

Yield of the maize grain [dt ha^{-1}] (KRUCZEK 2005d)

Studied factors	Years			Mean
	2001	2002	2003	
Hydrofoska 16; N/P = 1:0.44	98.7	66.6	101.6	89.0
Amofoska N:P = 1:1.74	100.6	65.5	102.6	89.6
Polifoska 8; N:P = 1:1.31	102.6	68.0	97.3	89.3
Ammonium phosphate N:P = 1:1.11	98.9	69.9	106.7	91.8
Superphosphate	98.9	65.3	104.0	89.4
$LSD_{0.05}$	n.s.	n.s.	n.s.	n.s.
Broadcast fertilization	97.7	66.8	100.8	88.4
In rows fertilization	102.2	67.4	104.0	91.2
$LSD_{0.05}$	2.6	n.s.	1.0	1.2

n.s. – no significant differences

Experiment 5. Response of medium early maize cultivars to fertilization method

In all the years of the study starter fertilization. in comparison to conventional broadcasting. significantly increased dry matter of aboveground parts of a single plant at the phase of 6–7 leaves (Table 12). On average for years (2004–2007) the greatest vigour of initial growth was found for hybrids Eurostar and Monumental. which differed significantly in terms of the level of this trait from hybrid Veritis. which dry matter of a single plant was the lowest. Hybrids tested in the experiment. when started fertilization was applied. had an increased dry matter in the initial period of plant development in comparison to broadcasting. Starter fertilization had an advantageous effect on grain yield. significantly increasing its level in comparison to broadcasting. on average for years and cultivars (Table 13). This information is of particular importance. since no significant interaction was observed for the method of fertilization with years of the study. A similar response to starter fertilization was found for all tested hybrids. Row application of ammonia phosphate significantly reduced grain moisture content in comparison to broadcasting (Table 14). A significant decrease in water content in grain as a result of starter application of fertilizer. in comparison to broadcasting was reported for hybrids Eurostar. Inagua. Energystar and LG 3226. It also needs to be stressed that the other hybrids responded similarly. although this difference was not confirmed statistically.

Table 12
The dry mass of above-ground parts of 1 plant in phase 6–7 leaves [g] (KRUCZEK and SKRZYPCZAK 2010)

Hybrids	Method of fertilization		Mean
	broadcast	in rows	
Eurostar	3.23	4.33	3.78
Monumental	3.38	4.16	3.77
PR 39H32	3.11	4.36	3.73
Menuet	3.39	3.88	3.63
System	3.09	4.12	3.61
Delitop	3.40	3.71	3.55
Inagua	2.68	3.73	3.21
LG 3226	2.90	3.38	3.14
Energystar	2.53	3.50	3.01
Veritis	2.54	3.37	2.96
Mean	3.02	3.85	–
$LSD_{0.05}$	for method of fertilization = 0.22		for hybrids = 0.78

Table 13

Yield of the maize grain [dt ha^{-1}] (KRUCZEK and SKRZYPCZAK 2010)

Hybrids	Method of fertilization		Mean
	broadcast	in rows	
Delitop	95.0	105.2	100.1
PR 39H32	90.0	94.1	92.0
Menuet	88.7	94.5	91.6
Veritis	87.9	92.4	90.2
Monumental	85.2	90.4	87.8
LG 3226	82.7	89.7	86.2
Eurostar	84.1	85.7	84.9
System	78.6	84.3	81.5
Energystar	79.0	83.5	81.2
Inagua	77.4	82.9	80.2
Mean	84.9	90.3	–
$LSD_{0.05}$	for method of fertilization = 1.5		for hybrids = 8.4

Table 14

Moisture content of grain at harvest [%] (KRUCZEK and SKRZYPCZAK 2010)

Hybrids	Method of fertilization		Mean
	broadcast	in rows	
Monumental	27.7	27.4	27.6
PR 39H32	27.4	26.8	27.1
Eurostar	27.3	26.4	26.9
Menuet	27.0	26.5	26.8
System	26.9	26.4	26.6
Inagua	27.1	26.1	26.6
Veritis	26.3	25.8	26.0
Energystar	26.3	25.0	25.6
Delitop	25.7	25.3	25.5
LG 3226	26.7	24.3	25.5
Mean	26.8	26.0	–
$LSD_{0.05}$	for method of fertilization = 0.3		for hybrids = 1.3

Conclusions

Presented results clearly indicate an advantageous effect of topical fertilization with nitrogen (N) and phosphorus (P) on growth. development and yielding in maize. This effect is particularly evident in the initial period of plant development. in which weather conditions in Poland are frequently stress factors for maize. An adverse course of weather conditions hinders

nutrient uptake by maize. mainly phosphorus. which leads to growth inhibition. Research results clearly indicate that this adverse phenomenon may be practically prevented by topical fertilization performed together with sowing. The positive effect of starter fertilization on maize in the initial period of vegetation is also reflected in its yielding. Yields of grain are significantly greater at fertilizer application simultaneous with sowing. than in the case of conventional broadcasting over the entire area of the field. A very important trait determining profitability of maize growing is also connected with grain moisture content at harvest. In all studies conducted at the Department of Agronomy the Poznan University of Life Sciences row application of fertilizers. in comparison to conventional broadcasting reduces water content in grain. Moreover. row fertilizer application makes it possible to reduce fertilization rates and extend the period of maize sowing. especially thanks to their acceleration. which is significant during periodical soil moisture deficits in early spring. Presented results are thus of considerable applicatory importance. which may improve economic results and organisation of maize growing in Poland.

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EVALUATION OF DIFFERENT POTATO FERTILIZATION REGIMES ON STARCH YIELD – PRODUCTION AND ECONOMIC ASPECTS

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K e y w o r d s: fertilization, starch, costs, profitability, potato.

A b s t r a c t

The article contains data concerning the production and economic aspects of producing starch from three starch potato cultivars, such as Adam, Pasja Pomorska and Ślęza. The considerations are based on an experiment conducted in 2008–2010 at the Experimental Station of the University of Warmia and Mazury in Olsztyn, situated in Bałcyny (N = 53°35'49''; E = 19°51'20,3'').

The study has shown that the cultivar Adam is the least economically useful starch potato, as it gives low starch yields, generates very high unit costs and presents the least favourable response to modifications in foliar fertilization that do not lead to the improvement of yielding. The lowest unit costs of producing starch occurred in the production of the potato cultivar Ślęza. Depending on the applied foliar fertilization treatments, the starch production unit costs decreased the most for the cultivar Pasja Pomorska, reaching the highest cost reduction level in the variant consisting of soil dressing *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) and foliar fertilization variant *a* – Basfoliar 12-4-6 [8 dm ha^{-1}].

PRODUKCYJNO-EKONOMICZNA OCENA WPŁYWU RÓŻNYCH SPOSOBÓW NAWOŻENIA ZIEMNIAKA NA WYDAJNOŚĆ SKROBI

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Abstrakt

W artykule ujęto dane dotyczące uwarunkowań produkcyjno-ekonomicznych skrobi trzech wybranych odmianach ziemniaka skrobiowego: Adam, Pasja Pomorska i Ślęza. Bazą były badania prowadzone w latach 2008–2010 w Stacji Doświadczalnej Uniwersytetu Warmińsko-Mazurskiego w Bałcynach (N = 53°35'49'; E = 19°51'20,3').

W badaniach wykazano, iż najmniej przydatną do uprawy w warunkach glebowo-klimatycznych Warmii, ze względu na najniższą wydajność skrobi, z punktu widzenia ekonomicznego, jest odmiana Adam, która generuje niskie plony skrobi, najwyższe koszty jednostkowe pozyskania oraz najmniej korzystnie reaguje na modyfikacje nawożenia dolistnego, nie skutkując poprawą plonowania. Najniższe koszty jednostkowe skrobi wystąpiły w produkcji ziemniaka odmiany Ślęza. W zależności od zastosowanych wariantów nawożenia dolistnego w odmianie Pasja Pomorska następowały największe spadki kosztu jednostkowego skrobi, który osiągnął najwyższą wartość redukcji kosztu w wariancie nawożenia doglebowego *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) oraz w nawożeniu dolistnym *a* – Basfoliar 12-4-6 [8 dm ha^{-1}].

Introduction

Both in Poland and across the world, dynamic changes are taking place in dedicating agricultural plantations to some innovative purposes. Traditional cultivation of plants for food and feeds is often replaced by growing energy or industrial crops. Such transformations affect many agricultural plants, and especially potato. Potato is a versatile crop plant, which has gained popularity all over the world. In 2014, the global potato production exceeded 358 million tons, which is the highest yield volume in history. For comparison, 328 million tons of potato were harvested in 2000, and since then the potato production has been increasing steadily. The biggest potato producer is Asia (over 43% of the world's production), followed by Europe (nearly 38%) (FAOSTAT 2016). In Poland, however, potato production has been on the decrease, mainly because of the diminishing total acreage planted with potatoes, which cannot be offset by the higher yields per ha (Table 1). This trend in the Polish potato

Table 1

Changes in acreage, yield and crops of potato in the years 1990–2014 in Poland

Years	Production area [thous. ha]	Yield [t ha^{-1}]	Harvest [mln t]
1991–1995	1694	16.1	27.34
1996–2000	1292	18.1	23.37
2001–2005	813	18.1	14.68
2006–2010	525	19.0	9.88
2011	406	23.0	9.36
2012	373	24.2	9.04
2013	346	21.0	7.29
2014	277	27.8	7.70

Source: based on NOWACKI (2015)

production is a consequence of the process of replacing smaller plantations by larger ones, where potato production is intensified. In the past, the most popular cultivation technology was a low-input system, preferred by smaller farmers. Meanwhile, the sustainable and intensive technologies have been gaining ground. The organic system is not very popular in potato production (NOWACKI 2012). The same tendencies are likely to continue in the following years (*Rolnictwo w latach 2000–2013*).

Table 2 collates information on changes in the use of harvested potatoes in Poland. Noteworthy is a very big increase in the amount oftubers dedicated to industrial processing, from 11.5% in the 2005/2006season to 25.2% in the 2015/2016 season, which has induced very big changes in the economic and production aspects of potato cultivation DZWONKOWSKI i in. 2015).

Table 2

Distribution of domestic potato yields [%]

Distribution targets							
Season	losses and damage	seed potatoes	animal feed	self-supply of farmer	sold for human consumption	industrial processing	export
2005/06	11.0	10.6	35.3	12.4	19.0	11.5	0.2
2015/16	7.7	8.6	15.2	15.6	26.9	25.2	0.8

Source: based on DZWONKOWSKI i in. (2015)

The total output of potato starch produced in the European Union is 1.95 million tons, of which 656,000 tons come from Germany. Germany is the biggest starch producer in the EU, followed by the Netherlands, France, Denmark and Poland (EMMANN et al. 2012). In 2015, the situation was slightly different, namely 28% of starch potato was produced in Germany, 24% in the Netherlands, 13% in Poland, 12% in Denmark and 11% in France (*Bundesverband...* 2016). In Germany, starch producers are mostly found in the following lands: Niedersachsen, Bayern and Brandenburg (UNIKA 2011). Many experts claim that the potato starch market will decrease down to the 80–85% of today's production output, which will anyway meet the demand (EMMANN et al. 2012). This tendency has been manifested by a decline in the acreage seeded with starch potato in Germany, which started back in 2001. Since that year, the total area of starch potato plantations has fallen from 95,000 ha to 86,000 ha (TOP AGRAR 2010). In 2015, the total area of fields allocated to starch potato cultivation in Germany declined to the record lowest level, i.e. to 52 796 ha (*Bundesverband...* 2016).The reason is the steadily decreasing subsidies allocated to starch potato production and processing (EMMANNET al. 2012). Until 2011,

starch producing companies could take advantage of higher prices, which allowed them to go through the transition period (DREETZ 2011). Potato production in Poland has been steadily declining, mainly due to the decreasing demand by agriculture and in response to the lower market demand. In 2015, potatoes were grown on 279.000 ha (MRIRW 2016).

The aim of this paper is to discuss the impact of different fertilization regimes applied to starch potato on the effectiveness and costs of starch production.

Materials and Methods

The results presented and discussed in this paper originate from a controlled field experiment set up at the Agricultural Research Station in Bałcyny (N = 53°35'49'; E = 19°51'20,3'') and conducted in 2008–2010. A multiple (repeated over years) three-factorial experiment with three replications was set up according to the method of random split-plot sub-blocks on grey-brown podsolic soil developed from boulder clay. The area of each plot planted with potatoes was 24 m^2, of which 18 m^2 was harvested.

Potatoes were planted in the last ten days of April. Tubers (class CA material) were planted at 40-cm intervals, in rows spaced at 62.5 cm, hence the plant density was 40.000 plants per hectare.

Each year, potatoes were grown in a field previously cropped with cereals, without organic fertilization. They were harvested at the full ripeness stage, in the last ten days of September. The cultivation treatments consisted of double earthing-up and several pesticide sprays. Dicotyledonous weeds were controlled with the herbicide AfalonDyspersyjny 450 S.C. dosed at 2 dm ha^{-1}. Potato blight was prevented by using the systemic fungicides Ridomil Gold MZ 68 WG 2 kg ha^{-1} and Tattoo C 750 SC 2 dm ha^{-1}, while surface acting preparations Antracol 70 WG 1.8 kg ha^{-1} and Gwarant 500 SC 2 dm ha^{-1} were appliedon a later date. Colorado beetle was controlled with the neonicotinoids Apacz 50 WG 40g ha^{-1}, Calypso 480 SC 0.08 dm ha^{-1} and Mospilan 20 SP 80 g ha^{-1}.

All calculations were made in line with the Polish agricultural bookkeeping methodology (GORAJ 2000), including the classical division into direct and indirect costs, which enabled us to calculate basic categories of costs and revenue according to the following scheme (AUGUSTYŃSKA-GRZYMEK et al. 2009):

1. Direct costs (DC).
2. Indirectcosts (IC).
3. Total costs (TC).
4. Unit productioncosts (UC).

The unit costs of tractors and machinery as well as the costs of conducting individual agrotechnical treatments were calculated according to the methods used at the Institute for Building, Mechanization and Electrification of Agriculture in Poland (MUZALEWSKI 2007). The calculations of agricultural machinery costs included the costs of using machines owned by the Experimental Station in Bałcyny: an URSUS C-385 tractor, URSUS C-360 tractor, John Derre 6930 tractor, Kverneland 7-farrow plough, Kverneland 5-farrow plough, drag spike-tooth harrow with 10 points, N-035 fertilizer spreader, S 227 plant seeder, P430/2weeder, Krukowiak ORP/2500/18/PHN sprayer, Anna potato combine, and an HW 6011 dump trailer. The analysis of economic effectiveness was conducted on the basis of the records of treatments and type of implements used in these treatments, as well as determined inputs of labour, power and inputs of materials. The hourly wage was set according to the remuneration system at the Experimental Station.

Starch yield. After harvest, the following were determined: total potato tuber yield, percentage content of starch in potato tubers (the weighing method on a Sengbusch weighing balance), and starch yield as a function of total yield and percentage starch content. Based on starch yields in particular fertilization variants, a starch yield increase was calculated relative to the zero variant h.

For the economic assessment, three-year average starch yields were taken as the main assessment criterion. The results were analyzed statistically using analysis of variance. The Tukey's test was applied to evaluate the inter-treatment variation, assuming the probability of error to be $p=0.05$. All analyses were accomplished with the help of STATISTICA 10® software.

The following factors were considered:

I. The first factor consisted of potato cultivars: Adam (medium early), Pasja Pomorska (medium late) and Ślęza (late).

II. The second factor included levels of soil fertilization:

A – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K),

B – 420 kg ha^{-1} NPK (120 N, 144 P, 156 K).

The soil fertilizing treatments were composed of potassium salt (60%), granulated triple superphosphate (46%) and ammonium saltpetre (34%), applied once prior to potato planting.

III. The third factor corresponded to foliar fertilization:

a – Basfoliar 12-4-6 [8 dm ha^{-1}],

b – ADOB Mn [4 dm ha^{-1}],

c – Solubor DF [2 dm ha^{-1}],

d – ADOB Mn + Solubor DF [2 + 1 dm ha^{-1}],

e – ADOB Mn + Basfoliar 12-4-6 [2 + 4 dm ha^{-1}],

f – Basfoliar 12-4-6 + Solubor DF[4 + 1 dm ha^{-1}],

g – Basfoliar 12-4-6 + ADOB Mn + Solubor DF [2.7 + 1.3 + 0.7 dm ha^{-1}],
h – control treatment – no foliar fertilization.

The foliar fertilizers were applied once, at the early inflorescence phase (BBCH 61].

Results and Discussion

Starch is a natural, renewable biopolymere, the demand for which arises from its use as raw material in production of beverages, sweets, fat reducing products, paper, cardboard paper, pharmaceuticals, textiles, feeds and many other products (FACHAGENTUR 2014). Technology-wise, the best raw material for making starch products is potato starch. The physical and chemical structure of potato starch makes it easily modifiable by various physical and chemical factors. However, it should be remembered that cereal starch can successfully compete with and virtually always replaces potato starch as raw material (DZWONKOWSKI 2007). In the future, the use of potato starch will depend on the costs of producing this sugar, at all stages of its manufacture, i.e. from the cultivation of starch plants, their transport and processing to the making of starch and any by-products.

Table 3 contains a complex specification of the effects of potato fertilization on the starch yield, depending on the variants of foliar and soil nutrition of the three cultivars analyzed in our experiment, the highest starch yields were produced by cv. Ślęza. Starch yields obtained from tubers of this cultivar in both soil fertilization variant *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) and variant *B* – 420 kg ha^{-1} NPK (120 N, 144 P, 156 K) were higher than from tubers of the other cultivars. The highest starch yield [9.18 mg ha^{-1}] was achieved in soil fertilization variant *B* – 420 kg ha^{-1} NPK (120 N, 144 P, 156 K) and foliar fertilization variant *f* – Basfoliar 12-4-6 + Solubor DF [4 + 1 dm ha^{-1}]. Slightly less starch, 9.15 mg ha^{-1}, was found in soil fertilization variant *B* – 420 kg ha^{-1} NPK (120 N, 144 P, 156 K) and foliar fertilization variant *g* – Basfoliar 12-4-6 + ADOB Mn + Solubor DF [2.7 + 1.3 + 0.7 dm ha^{-1}]. The lowest productivity was achieved by cv. Adam, which gave the lowest starch yield of 5.08 mg ha^{-1} in soil fertilization variant *A*– 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) and foliar fertilization variant *c* – Solubor DF [2 dm ha^{-1}]. The data in table 3 demonstrate that the cultivars responded differently to modifications in foliar fertilization, but it was only cv. Pasja Pomorska grown in soil fertilization variant *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) that produced a higher starch yield when supplied foliar fertilization according to variant *a* – Basfoliar 12-4-6 [8 dm ha^{-1}].

Table 3

Effect of different potato fertilization variants on starch yield

Specification	Cultivar	Variants of foliar fertilization*	Variants of soil fertilization							
			a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
Starch yield [Mg ha^{-1}]	Adam	*A*	5.38	5.21	5.08	5.39	5.34	5.5	5.28	5.52
Starch yield gain** [Mg ha^{-1}]			-0.14	-0.31	-0.44	-0.13	-0.18	-0.02	-0.24	–
Starch yield [Mg ha^{-1}]		*B*	5.62	5.48	5.75	5.76	5.51	5.88	5.79	5.71
Starch yield gain** [Mg ha^{-1}]			-0.09	-0.23	0.04	0.05	-0.20	0.17	0.08	–
Starch yield [Mg ha^{-1}]	Pasja Pomorska	*A*	7.60	7.19	7.33	7.36	7.28	7.49	7.45	7.15
Starch yield gain** [Mg ha^{-1}]			0.45	0.04	0.18	0.21	0.13	0.34	0.30	–
Starch yield [Mg ha^{-1}]		*B*	7.03	6.77	7.28	7.46	6.85	7.12	6.99	7.38
Starch yield gain** [Mg ha^{-1}]			-0.35	-0.61	-0.10	0.08	-0.53	-0.26	-0.39	–
Starch yield [Mg ha^{-1}]	Ślęza	*A*	8.75	8.32	8.86	8.57	9.06	8.95	8.58	8.61
Starch yield gain** [Mg ha^{-1}]			0.14	-0.29	0.25	-0.04	0.45	0.34	-0.03	–
Starch yield [Mg ha^{-1}]		*B*	8.84	8.78	8.6	8.99	8.78	9.18	9.15	9.10
Starch yield gain** [Mg ha^{-1}]			-0.26	-0.32	-0.50	-0.11	-0.32	0.08	0.05	–
$LSD_{(0.05)}$ of cultivars – 0.17; cultivar x soil fertilization – 0.25; other factors and non-significant interactions										

* details in the Methods, ** relative to technology *h*

Production costs are generated by many production factors, most of which can be extensively manipulated by theproducer, except the weather conditions. In the reported experiment, the total costs of potato cultivation were in a range of 6.4 thousand PLN ha^{-1} to over 7.4 thousand PLN ha^{-1} (Table 4). The difference was a result of the differences in the price of seed potatoes and the applied soil fertilization variant. Economically speaking, however, the unit costs of producing the raw material are much more important. Our calculations proved that the lowest unit costs of producing starch were obtained for c. Ślęza grown in soil fertilization variant *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K), where they equalled 797 zł Mg $^{-1}$. The highest unit production costs were generated by growing cv. Adam in fertilization variant *B* – 420 kg ha^{-1} NPK (120 N, 144 P, 156 K), where they were slightly higher than in variant *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K). The unit production costs of growing the third tested cultivar, Pasja Pomorska, reached the same level as the production costs of the average starch yield for the whole experiment. In general, the total costs of potato cultivation(materials seeds, mineral fertilization, crop protection, cost of operating tractors and machines, labor costs, agricultural tax, other indirect costs), depending on the type of production, are high and range from 8.5 to nearly 17 thousand PLN ha^{-1} (NOWACKI 2015).

Table 4
Costs of starch production from selected starch potato cultivars including soil fertilization [PLN ha^{-1}]

Cultivar	Adam		Pasja Pomorska		Ślęza		Średnio	
Fertilizaion variant	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Starch yield [Mg ha^{-1}]	5.3	5.7	7.4	7.1	8.7	8.9	7.1	7.2
Direct costs in total:	3513.77	3974.77	3729.77	4190.77	4053.77	4520.83	3765.77	4229.34
– seed potatoes	2052.00	2052.00	2268.00	2268.00	2592.00	2592.00	2304.00	2304.00
– mineral fertilization	917.77	1378.77	917.77	1378.77	917.77	1384.83	917.77	1381.34
– plant protection	544.00							
Indirect costs in total:	2888.14							
– labour of tractors and machines	2229.58							
– labour in puts	271.00							
– agricultural tax	125.00							
– other indirect costs (+10%)	262.56							
Total costs	6401.91	6862.91	6617.91	7078.91	6941.91	7408.91	6653.91	7117.48
Unit costs PLN/ Mg $^{-1}$	1198.92	1206.15	899.27	995.68	797.01	829.76	931.97	983.14

Table 5
Specification of unit costs of the starch production in different fertilization variants of selected starch potato cultivars [PLN Mg $^{-1}$]

Soil fertilizaion variant	Foliar fertilization variants								
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	Mean
Adam									
A	1190.4	1232.0	1256.6	1187.5	1200.7	1162.5	1212.4	1146.6	1198.9
B	1221.6	1255.4	1190.3	1191.2	1247.3	1165.7	1185.2	1189.1	1206.1
Mean	1206.0	1243.7	1223.5	1189.4	1224.0	1164.1	1198.8	1167.9	1202.5
Pasja Pomorska									
A	871.1	922.7	900.3	899.0	910.4	882.4	888.3	915.4	899.2
B	1007.3	1048.1	969.8	948.7	1034.8	993.0	1012.7	949.3	995.6
Mean	939.2	985.4	935.1	923.9	972.6	937.7	950.5	932.4	947.4
Ślęza									
A	793.7	836.4	781.4	809.9	767.3	774.7	809.0	797.8	797.0
B	837.7	845.0	858.7	823.3	844.2	805.5	809.0	805.5	829.7
Mean	815.7	840.7	820.0	816.6	805.8	790.1	809.0	801.6	813.6
Mean for cultivars									
A	919.4	965.3	935.9	935.7	921.6	909.1	935.8	928.2	931.9
B	1007.3	1048.1	969.8	948.7	1034.8	993.0	1012.7	949.3	983.1
Mean	963.4	1006.7	952.9	942.2	978.2	951.1	974.2	938.8	985.5

Table 5 contains detailed data regarding the unit starch production costs, including the division into the variants of soil and foliar fertilization. The three cultivars responded in a highly diverse manner to the individual variants of fertilization. The lowest average unit cost was calculated for cv. Ślęza. The same cultivar was distinguished by attaining the lowest unit cost of all computed values, which equaled 767.3 zł Mg $^{-1}$ in soil fertilization variant *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) and foliar fertilization *e* – ADOB Mn + Basfoliar 12-4-6 [2 + 4 dm ha^{-1}].

Economically speaking, it is important to know how individual factors affect the unit costs. The following set of data (Table 6) shows differences in the levels of unit costs calculated per Mg $^{-1}$ of starch. Large differences can be seen between the unit costs derived for the control and a specific fertilization variant among the soil and foliar fertilization techniques. The biggest decrease

Table 6

Specification of differences in unit costs of starch production between the control and foliar fertilization at a given soil fertilization variant for selected starch potato cultivars [PLN Mg $^{-1}$]

Soil fertilizaion variant	Foliar fertilization variants								
	a	*b*	*c*	*d*	*e*	*f*	*g*	control	mean
Adam									
A	43.9	85.4	110.0	40.9	54.1	15.9	65.8	0.0	52.3
B	32.5	66.2	1.2	2.1	58.1	-23.4	-3.9	0.0	17.0
Mean	38.2	75.8	55.6	21.5	56.1	-3.8	31.0	0.0	34.6
Pasja Pomorska									
A	-44.3	7.3	-15.1	16.4	-5.0	-32.9	-27.1	0.0	16.2
B	58.0	98.7	20.5	-0.6	85.5	43.7	63.3	0.0	46.3
Mean	6.9	53.0	2.7	-8.5	40.2	5.4	18.1	0.0	15.0
Ślęza									
A	-4.1	38.6	-16.4	12.1	-30.5	-23.1	11.2	0.0	-0.8
B	32.2	39.5	53.2	17.8	38.7	0.0	3.5	0.0	23.5
Mean	14.0	39.1	18.4	14.9	4.1	-11.6	7.4	0.0	11.4
Mean for cultivars									
A	-8.8	37.1	7.7	7.4	-6.6	-19.1	7.6	0.0	3.7
B	58.0	98.7	20.5	-0.6	85.5	43.7	63.3	0.0	46.3
Mean	24.6	67.9	14.1	3.4	39.5	12.3	35.5	0.0	25.0

in unit costs versus the control was found for cv. Pasja Pomorska in soil fertilization variant *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) and foliar fertilization *a* – Basfoliar 12-4-6 [8 dm ha^{-1}], where it reached over 44 PLN Mg $^{-1}$. In the other fertilization variants applied to the same cultivar, a decline in unit costs was also quite evident, ranging from 27 to 32 PLN Mg $^{-1}$, which in

both cases occurred in soil fertilization variant *A* at 280 kg ha^{-1} NPK (80 N, 80 P, 120 K). Among the other cultivars, unit costs were decreased for cv. Ślęza in the same soil fertilization variant as reported for cv. Pasja Pomorska, i.e. variant A 280 kg ha^{-1} NPK (80 N, 80 P, 120 K). The unit costs were particularly lowered when combined with foliar fertilization variant *e* – ADOB Mn + Basfoliar 12-4-6 [2 + 4 dm ha^{-1}]. In this combination, the decrease was over 30 PLN Mg $^{-1}$ compared to the control unit cost.

Although starch production in Poland encounters many obstacles, the starch production industry – after a period of potato starch production quotas in the EU (until 2013) – is now overcoming the crisis. Moreover, the rise in prices for starch on the global market can stimulate the production of potato starch in Poland and contribute to improved profitability of starch potato plantations (NOWACKI 2015). Also, the subsidies to starch potato cultivation in the amount of 400 euro/ha planned to be paid until 2020 will strengthen the competitive advantage of potato starch production. This situation should level off continual fluctuations on the starch potato market, also because of the obligatory contracts, which many potato farmers see as an opportunity (KOROLEWICZ 2015). The production of starch potato is most heavily burdened by the costs of using tractors and other machinery, which in our experiment accounted for 30 to 34% of the total production costs and reached similar values in other research (TURSKA 2014). The second biggest cost is represented by the purchase of seed potatoes, which can reach from 53.9 to 56.6% of direct cost (SKARŻYŃSKA 2010). In our experiment, this cost was around 58 to 64% of direct costs, but it can be lowered if farmers buy larger batches of seed potatoes, or make purchases as formal or even informal consortia of producers.

From the point of view of a producer, alternative costs play an important role in the "setting-up" of production. Comparison of costs and economic profits as well as the production management aspects related to the production of starch potato and other agricultural crops enable the farmer to make a good decision. Regarding the costs of setting up and running a plantation of starch potatoes, they are 3- to 4-fold as high as the analogous costs of starting a plantation of such crops as winter triticale, which in a study of Dubis et al. (DUBIS et al. 2015) equalled 2.2–2.5 thousand PLN ha^{-1}. Compared with spring barley, the total costs of starch potato cultivation were 4- to 5-fold higher (ŻUK-GOŁASZEWSKA et al. 2013). On the other hand, they were just less than 100% higher than the costs related to the cultivation of winter oilseed rape (GUGAŁA et al. 2015). The actual results depend on the intensity of plant production, as this factor determines costs of the inputs. What certainly distinguishes the cultivation of starch potato from the production of the other crops mentioned above is the input of the labour of tractors and machines, which – as already suggested – is decisive for the profitability of starch potato production.

Nowacki (NOWACKI 2012) concluded that potato production must be intensified because any extensive system of its production generates losses. In integrated or organic systems of production, the outcome will be profitable for the farmer only when higher prices for the produced potatoes are guaranteed. Irrigation may become an important treatment in potato production, as it is able to generate a yield increase of as much as 100%. It needs to be added that 1 mm of water used for irrigation raises the tuber yield by around 70.1 kg ha^{-1} (RĘBARZ and BORÓWCZAK 2006).

The prospects of starch potato and potato starch production in Poland will mostly depend on the pressure produced by cereal starch production and on the system of subsidies on the cereals market and subsidies allocated to starch (REMBEZA 2002). With prices of cereals falling, the market prices for potatoes must be compensated for by higher subsidies dedicated to starch. Otherwise, potato starch production will not be profitable.

Conclusions

The experimental results lead to the conclusion that the tested starch potato cultivars responded in a highly differentiated way to the treatments, in terms of both starch yields and the associated economic output. The analysis of the data derived from the experiment substantiates the following conclusions:

– the highest starch yields in the field experiment were obtained from cv. Ślęza; they were about 3 Mg ha^{-1} higher than the starch yield produced by the lowest-yielding cultivar Adam;

– the cultivar Pasja Pomorska in the variant with soil fertilization *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) generated the highest increase in starch yield, which was the highest in foliar fertilization variant *a* – Basfoliar 12-4-6 [8 dm ha^{-1}];

– economically speaking, the cultivar Adam was the least useful one, as it produces very low starch yields, generates high unit production costs and presents the least favourable response to modifications in foliar fertilization;

– the lowest unit costs of starch production were calculated for cv. Ślęza, and this result repeated in nearly all fertilization variants;

– in response to the applied foliar fertilization variants, cv. Pasja Pomorska demonstrated economically highly desirable decrease in the unit costs of starch production, which was the biggest in soil fertilization variant *A* – 280 kg ha^{-1} NPK (80 N, 80 P, 120 K) and foliar fertilization *a* – Basfoliar 12-4-6 [8 dm ha^{-1}].

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CHEMICAL COMPOSITION OF THE COLOSTRUM AND MILK OF SOWS FED DIETS CONTAINING NAKED OATS

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K e y w o r d s: sow nutrition, milk composition, lactoglobulins, milk fatty acids.

A b s t r a c t

The aim of the study was to determine the chemical composition of the colostrum and milk of sows fed diets containing naked oats. The study was carried out on 45 Polish Landrace sows assigned to three groups, 2 experimental and one control, with 15 individuals in each group. In the late gestation period the sows were also fed a diet including naked oats. The ration fed to the experimental groups contained 20% (D_1) and 40% (D_2) naked oats of the *Akt* variety. The chemical composition of the colostrum and milk of the sows was tested during the first, second and third lactation. Colostrum and milk for chemical analysis were collected on days 1, 7 and 21 of lactation (basic composition) following prior administration of 2 ml of oxytocin. The level of lactoglobulins in the colostrum and milk was determined on days 1 and 7 of lactation, and the fatty acid profile on day 7. On both days 7 and 21 of lactation the milk of the sows in the experimental groups had a higher percentage of fat than in the control. The milk of the experimental sows also contained higher percentages of linoleic and linolenic acids. Statistically significant differences ($P < 0.01$) were shown in the percentages of these acids in the milk of sows during each lactation between the D_2 groups and the control groups.

SKŁAD CHEMICZNY SIARY I MLEKA LOCH ŻYWIONYCH DIETĄ Z UDZIAŁEM OWSA NAGIEGO

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S ł o w a k l u c z o w e: żywienie loch, skład mleka, laktoglobuliny, kwasy tłuszczowe mleka.

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A b s t r a k t

Celem pracy było określenie składu chemicznego siary i mleka loch żywionych dietą zawierającą owies nagi. Badanie przeprowadzono na 45 lochach rasy polskiej białej zwisłouchej, przydzielonych do trzech grup: dwóch doświadczalnych i jednej kontrolnej – po 15 osobników w każdej. W okresie wysokiej ciąży badane lochy były również żywione dietą z udziałem owsa nagiego. Mieszanka podawana grupom doświadczalnym zawierała 20% (D_1) i 40% (D_2) owsa nagiego odmiany *Akt*. Skład chemiczny siary i mleka loch badano w trzech kolejnych laktacjach, począwszy od pierwszej do trzeciej laktacji włącznie. Siarę i mleko do analiz chemicznych pobierano w 1., 7. i 21. dniu laktacji (skład podstawowy) po uprzednim podaniu 2 ml oksytocyny. Poziom laktoglobulin w siarze i mleku określono w 1. i 7. dniu laktacji, natomiast profil kwasów tłuszczowych w 7. dniu. W obu dniach laktacji (w 7. i 21.) mleko loch z grup eksperymentalnych wykazywało wyższy odsetek tłuszczu niż z grupy kontrolnej. Mleko loch z grup doświadczalnych zawierało również wyższy odsetek kwasu linolowego i linolenowego. Wykazano statystycznie istotne różnice ($P < 0{,}01$) w procentowej zawartości tych kwasów w mleku loch w poszczególnych laktacjach między grupami D_2 a grupami kontrolnymi.

Introduction

Milk production by sows is one of the most important performance characteristics affecting the development of piglets. Changes in the chemical composition and the milk yield of sow are the result of genetic (WALKIEWICZ at al. 2000) and environmental factors (MIGDAŁ and KACZMARCZYK 1990, BUCZYŃSKI at al. 2003), but depend mainly on nutrition (KOKETSU at al. 1996b, MIGDAŁ 1996, KIM and EASTER 2001, WOLTER at al. 2002). KIM and EASTER (2001) note that complete fulfilment of the nutritional needs of sows during pregnancy and lactation are reflected in a substantial increase in the content of protein, amino acids and fat in their milk, particularly in the early stage of lactation. Many authors (BOYD at al. 1979, MOSER and LEVIS 1981, PETTIGREW 1981) confirm the beneficial effect of adding fats to feed rations for sows on lipid content in the colostrum and milk and on milk yield. When vegetable oils are included in the diet of sows, either during advanced pregnancy or during the entire pregnancy and lactation, fat content in the colostrum and milk increases and the ratio of essential unsaturated fatty acids to saturated fatty acids in the fat is more beneficial to piglets (COFFEY at al. 1994, MIGDAŁ 1996). KOKETSU et al. (1996a) used high-energy feed rations during lactation and observed increased secretion of insulin, glucose and luteotropic hormone (LH), both during lactation and after the piglets were weaned.

One of the valuable components of feed rations for pigs is oats. Due to the genetic lack of a hull, naked oats have a different chemical composition from that of hulled oats. The greatest differences are in the content of fibre, protein and fat – the components that primarily determine the nutritional and energy value of fodder (PELTONEN-SAINIO 1997, CZUBASZEK 2003, DUBIS and BUDZYŃSKI 2003). Naked oats have higher energy value and protein content

than other cereals regarded as the most beneficial for feeding monogastric animals (PETKOV at al. 2001). An important component of oats with and without hulls is fat, the content of which ranges from 6% to 8% of dry matter (PIECH at al. 2003, PISULEWSKA at al. 2011). The fat of naked oats is dominated by unsaturated fatty acids (UFA), which account for 80% of the fat (PISULEWSKA at al. 1999). The lipids in oats have been found to contain compounds with strong antioxidant properties, such as tocopherols, ferulic acid, caffeic acid, polyphenolic compounds, their esters and amides, alkylphenols, flavonoids and avenanthramides (PETERSON 2001). Studies by STASIAK et al. (2000), MAZUR and STASIAK (2006) found that feed rations containing naked oats had a beneficial effect on reproductive performance indicators in gilts and sows.

The aim of the study was to determine the chemical composition of the colostrum and milk of sows fed diets containing naked oats.

Material and Methods

The study was carried out on 45 Polish Landrace sows assigned to three groups, 2 experimental and one control, with 15 individuals in each group. The sows were fed complete feed rations in amounts consistent with the requirements given in *Nutrient requirements of pigs* (1993). The feed ration fed to the experimental groups contained 20% (D_1) and 40% (D_2) naked oats of the *Akt* variety. In the late gestation period the sows were also fed a diet including naked oats. The chemical composition of naked oat was determined before the experiment on the animals was begun. The following were determined in the samples:

– content of crude protein, ether extract, crude ash, and crude fibre according to AOAC (2000);

– content of mineral nutrients Ca and Na by atomic absorption spectroscopy (ASA) and total phosphorus according to FISKE and SUBBAROW (1925);

– protein amino acid content by ion-exchange chromatography in an automatic amino acid analyser;

– fatty acid composition by gas chromatography using a chromatograph (Varian GC3800). The fatty acid profile of the fat of the naked oats was as follows: unsaturated fatty acids – 81.17%, including monounsaturated fatty acids – 44.45% (mainly oleic acid) and polyunsaturated fatty acids – 36.72% (mainly linoleic acid – 35.27% and linolenic acid – 1.41%). The composition and nutritional value of the diet fed to the sows during pregnancy and lactation is presented in Table 1.

Table 1

The composition and nutritive value of the diets for pregnant and lactating sows

Feed [%]	Before 90th day of gestation			Lactation		
	K	D_1	D_2	*K*	D_1	D_2
Naked oats meal	–	20.00	40.00	–	20.00	40.00
Wheat meal	40.00	20.00	–	40.00	20.00	–
Barley meal	48.40	48.45	48,50	40.00	40.05	40.10
Soybean meal	9.00	9.00	9.00	17.00	17.00	17.00
2-Ca phosphate	0.90	0.90	0.90	1.00	1.00	1.00
Fodder chalk	1.30	1.30	1.30	1.30	1.30	1.30
Premixture L-lysine 50%	0.10	0.05	–	0.30	0.25	0.20
NaCl	0.30	0.30	0.30	0.40	0.40	0.40
Calculated analysis [g kg^{-1}]:						
EM MJ	12.75	12.89	13.03	12.72	12.87	13.01
crude protein [g]	136.32	137.18	138.03	159.00	159.85	160.71
crude fat [g]	20.32	30.17	39.98	20.39	30.24	40.10
lysine [g]	5.96	5.97	5.98	8.56	8.57	8.58
methionine+cystine [g]	4.84	4.95	5.06	5.40	5.51	5.62
Ca [g]	7.78	7.73	7.69	8.53	8.48	8.43
P [g]	5.55	5.64	5.73	6.09	6.18	6.27
Na [g]	1.35	1.36	1.37	1.77	1.78	1.79

The chemical composition of the colostrum and milk of the sows was tested during the first, second and third lactation. Colostrum and milk for chemical analysis were collected on days 1, 7 and 21 of lactation (basic composition) following prior administration of 2 ml of oxytocin. The level of lactoglobulins in the colostrum and milk was determined on days 1 and 7 of lactation, and the fatty acid profile on day 7. The samples collected were stored at -20°C. The percentage of fat, protein and lactose were determined in a Milko-Scan infrared milk analyser. The concentration of immunoglobulin (IgG) was determined by radial immunodiffusion (RID) with the Binding Site RID kit manufactured by the British company Binding Site Limited. The content of fatty acids was determined by gas chromatography.

Statistical analysis of the results was carried out using one-way analysis of variance (effect of group). The tables present mean values for the characteristics tested and the standard deviation. Differences between means from each group were tested by Duncan's range test.

Results and Discussion

The chemical composition of the colostrum and milk of sows fed different diets is presented in Table 2. The amount of protein in the colostrum and milk of the sows showed little variation between groups. No statistically significant

differences were noted for the traits analysed. The colostrum collected on the first day of the third lactation contained somewhat more protein and lactoglobulin (group D_2 – 12.38% and 106.42 mg ml^{-1}). The content of milk protein in sows milk at 7 and 21 days of lactation was higher than 5%. According to studies by MIGDAŁ and KACZMARCZYK (1990) and COFFEY et al. (1982), milk contains 5–6% proteins, which is in agreement with the values obtained in the present study.

Table 2

Chemical composition of the colostrum and milk of sows (mean ± SD)

Lactation number	Sampling date	Experimental groups	Total protein [%]	Fat [%]	Lactose [%]	Lactoglobulins [mg ml^{-1}]
I	1st day	K	10.90 ± 0.95	4.52 ± 0.50	3.42 ± 0.37	94.21 ± 7.11
		D_1	11.00 ± 1.01	4.71 ± 0.59	3.31 ± 0.30	94.50 ± 7.54
		D_2	11.24 ± 0.98	4.97 ± 0.69	3.28 ± 0.32	95.82 ± 8.43
	7th day	K	5.37 ± 0.48	6.59 ± 0.73	5.01 ± 0.44	0.95 ± 0.25
		D_1	5.26 ± 0.46	7.00 ± 0.90	5.10 ± 0.42	1.10 ± 0.21
		D_2	5.45 ± 0.52	7.17 ± 0.81	4.90 ± 0.43	1.09 ± 0.16
	21st day	K	5.19 ± 0.42	6.00 ± 0.79	5.10 ± 0.37	–
		D_1	5.17 ± 0.45	6.05 ± 0.84	5.07 ± 0.36	–
		D_2	5.31 ± 0.48	6.30 ± 0.80	5.00 ± 0.39	–
II	1st day	K	12.00 ± 1.02	5.20 ± 0.95	3.35 ± 0.35	99.87 ± 9.43
		D_1	11.93 ± 1.07	5.39 ± 0.90	3.21 ± 0.32	100.22 ± 9.94
		D_2	12.16 ± 1.00	5.58 ± 0.85	3.14 ± 0.30	101.76 ± 10.14
	7th day	K	5.32 ± 0.44	7.00 ± 0.85	5.00 ± 0.41	1.10 ± 0.26
		D_1	5.42 ± 0.43	7.14 ± 0.81	4.90 ± 0.39	1.27 ± 0.23
		D_2	5.48 ± 0.49	7.41 ± 0.79	4.85 ± 0.43	1.24 ± 0.20
	21st day	K	5.26 ± 0.48	6.15 ± 0.71	5.20 ± 0.34	–
		D_1	5.35 ± 0.46	6.31 ± 0.72	5.10 ± 0.36	–
		D_2	5.55 ± 0.44	6.50 ± 0.78	4.80 ± 0.37	–
III	1st day	K	12.10 ± 1.10	5.49 ± 0.85	3.24 ± 0.33	100.40 ± 8.94
		D_1	12.21 ± 1.09	5.64 ± 0.79	3.11 ± 0.30	104.51 ± 9.67
		D_2	12.38 ± 1.04	5.81 ± 0.84	3.03 ± 0.27	106.42 ± 10.34
	7th day	K	5.42 ± 0.48	6.95 ± 0.82	4.96 ± 0.41	1.35 ± 0.37
		D_1	5.32 ± 0.47	7.01 ± 0.84	5.05 ± 0.40	1.39 ± 0.31
		D_2	5.48 ± 0.50	7.22 ± 0.83	4.84 ± 0.39	1.42 ± 0.24
	21st day	K	5.15 ± 0.42	5.90 ± 0.79	5.21 ± 0.39	–
		D_1	5.20 ± 0.40	6.36 ± 0.80	5.04 ± 0.30	–
		D_2	5.28 ± 0.43	6.45 ± 0.81	5.05 ± 0.31	–

Fat content varied considerably between groups. The milk of the sows in the experimental groups contained more fat than the milk from the control on both the 7th and 21st days of lactation. Fat content was highest in group D_2, ranging on day 7 from 7.16% (first lactation) to 7.41% (second lactation), while on day 21 it ranged from 6.30% (first lactation) to 6.50% (second lactation).

The transition from colostrum to milk causes the level of protein to fall and that of fat and lactose to rise (MIGDAŁ and KACZMARCZYK 1990). This also confirms our findings.

Lactoglobulins noted an upward trend in the colostrum of sows fed a diet containing oats naked. The highest values included colostrums from group D_2 (101.76 – 106.42 mg ml^{-1}) in second and third lactations. BLAND et al. (2003) and RZĄSA (2007) found that colostrum contained on average 61–93 g l^{-1} IgG. The level of IgG in the colostrum of the sows in the present study should be regarded as very good.

The fatty acid profile of the milk lipids on day 7 of lactation is presented in Table 3. Among saturated fatty acids the highest proportion was that of palmitic acid – from 29.30% to 31.84%. The highest content of this acid, 31.84%, was found in the milk from the first lactation of the sows of the control group. The percentage of saturated fatty acids in the milk was highest in the control, at 41.21%. Unsaturated fatty acids were predominant in the total pool of analysed fatty acids. The highest percentage of oleic acid, 40.18%, was found in the milk of the group D_2 sows during their third lactation. Analysis of the results presented in Table 3 reveals higher percentages of this fatty acid in the experimental groups in which the sows were fed diets containing naked oats. The milk of the experimental sows also contained higher percentages of linoleic

Table 3

The fatty acid profile of the milk lipids on the 7th day of lactation of sows

Fatty acids	I Lactation			II Lactation			III Lactation		
	K	D_1	D_2	*K*	D_1	D_2	*K*	D_1	D_2
SFA:									
Lauric $C_{12:0}$	0.35	0.33	0.30	0.33	0.31	0.29	0.29	0.28	0.26
Myristic $C_{14:0}$	3.22	3.05	2.99	3.15	3.08	2.86	3.00	2.95	2.80
Pentadecanoic $C_{15:0}$	0.10	0.10	0.09	0.09	0.09	0.08	0.10	0.08	0.08
Palmitic $C_{16:0}$	31.84	31.42	30.40	31.65	31.05	30.02	31.38	30.74	29.81
Margaric $C_{17:0}$	0.49	0.45	0.42	0.45	0.43	0.41	0.47	0.43	0.41
Stearic $C_{18:0}$	5.01	5.08	4.97	4.95	5.12	5.01	5.00	5.14	5.06
Arachidic $C_{20:0}$	0.20	0.17	0.15	0.19	0.17	0.14	0.18	0.17	0.15
Total SFA	41.21	40.60	39.32	40.81	40.25	38.81	40.42	39.79	38.57
UFA:									
Myristooleic $C_{14:1}$	0.20	0.16	0.17	0.18	0.15	0.16	0.19	0.17	0.18
Palmitooleic $C_{16:1}$	10.15	9.80	9.72	10.02	9.76	9.86	10.20	9.92	9.81
Oleic $C_{18:1}$	38.52	38.79	39.75	38.83	39.10	39.99	38.92	39.27	40.18
Linoleic $C_{18:2}$	7.98^{Bb}	8.61^{a}	8.91^{A}	8.25^{B}	8.65	9.01^{A}	8.31^{B}	8.74	9.10^{A}
Linolenic $C_{18:3}$	0.36^{B}	0.47	0.49^{A}	0.37^{B}	0.48	5.52^{A}	0.40^{B}	0.49	0.50^{A}
Eicosenoic $C_{20:1}$	0.32	0.31	0.34	0.31	0.32	0.33	0.30	0.33	0.32
Cis11, 14-eicosenoic $C_{20:2}$	0.37	0.36	0.38	0.36	0.38	0.39	0.36	0.38	0.39
Arachidonic $C_{20:4}$	0.89	0.90	0.92	0.87	0.91	0.93	0.90	0.91	0.95
Total UFA	58.79	59.40	60.68	59.19	59.75	61.19	59.58	60.21	61.43

Means within a row with no common letters (*A*,*B*) differ significantly at $p \leq 0.01$
Means within a row with no common letters (*a*,*b*) differ significantly at $p \leq 0.05$

and linolenic acids. Statistically significant differences ($P < 0.01$) were shown in the percentages of these acids in the milk of sows during each lactation between the D_2 group and the control treatment.

The quantity and quality of milk produced by sows determines the health condition and body weight of their piglets and the number of piglets weaned per litter. Changes in the amount of milk produced by the sow and in its chemical composition depend mainly on nutrition (WIELBO 1995, MIGDAŁ 1996, KIM and EASTER 2001, PIETRAS and BAROWICZ 2002). DARRAGH and MOUGHAN (1998) report that the nutrients contained in milk remain at a constant level (with slight fluctuations in the case of optimal nutrition), which remains unchanged even when the level of one of the components is increased in the feed. According to REKIEL (2003), the level of nutrients in milk may increase only when an optimal diet is introduced for sows that are in poor condition or are inadequately nourished during lactation. Kim and EASTER (2001) state that complete fulfilment of nutritional needs during pregnancy and lactation leads to an increase in the content of protein, amino acids and fat in the milk of sows, particularly in the early stage of lactation. The chemical composition of colostrum and milk also depends on the stage of lactation, litter number, and number of piglets in the litter (BELSTRA at al. 1999, HODBOD and ZEMAN 2001, KIM and EASTER 2001). Milk composition does not stabilize until the second week of lactation (CSAPO et al. 1996). The albumin fraction of whey proteins in colostrum contains immunoglobulins IgG, IgA and IgM. The basic antibodies of colostrum are immunoglobulins G(IgG) (BLECHA 1998), which determine passive immunity in piglets. Piglets fed colostrum with a higher concentration of immune lactoglobulins are more resistant to post-natal stress, have a higher survival rate and a faster growth rate. Research has been conducted for many years aimed at developing feeding systems that enhance immunity, e.g. the use of immune proteins as a feed supplement to prevent diarrhoea in piglets (STEFANIAK 2006) or administration of immunostimulants to pregnant sows to improve the immune parameters of colostrum (KRAKOWSKI at al. 1999). Fat content in the milk was highly variable, and somewhat higher in the experimental groups. MIGDAŁ (1996) found that as the amount of fat consumed by the sow increases, the amount of fat secreted by the sow increases as well. BUCZYŃSKI et al. (2003) determined that piglets consuming milk with higher fat content had higher body weight on their 21st day of life. BAIDOO et al. (1992) also found that high-energy feed rations had a beneficial effect on the chemical composition of sows; milk. The addition of fat caused an increase in its content in the milk of the sows, which in turn positively affected the development of the piglets.

Milk fat is the most concentrated source of energy, hence the importance of its fatty acid composition, which determines its nutritional value (MIGDAŁ

1996). The present study showed a slightly higher percentage of unsaturated fatty acids, including essential ones, in the milk of sows from the experimental groups in comparison with the control. MIGDAŁ (1996) found a higher concentration of unsaturated fatty acids in the milk of sows whose feed rations were supplemented with rapeseed oil in comparison with those receiving the feed without oil. Similar results were obtained by WIELBO (1995) and VAN DEN BRAND et al. (2000). High content of polyunsaturated fatty acids in the colostrum and milk of sows receiving vegetable oil in their diet had a beneficial effect on the growth and development of their piglets (MIGDAŁ 1996, BABINSZKY 1998, PIETRAS and BAROWICZ 2002, BAROWICZ et al. 2003).

Conclusions

There were no significant differences in the amount of protein in colostrum and sows milk from control and experimental groups. Colostrum and milk of sows fed a diet involving naked oats had a slightly higher percentage of protein and fat.

Unsaturated fatty acids constituted the highest proportion of the lipid fraction of the milk on the 7th day of lactation. The level of these acids was highest in the group of sows fed a ration with 40% naked oats. A highly significant difference was noted in the concentrations of linoleic and linolenic acids between the control and D_2 groups in three successive lactations. The 40% of dietary inclusion of naked oats contributed to the highest level of UFA in milk and improved its nutritive value.

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ENVIRONMENTAL INFLUENCE OF CULTURAL MEDIUM ON BIOHERBICIDAL ACTIVITIES OF *PSEUDOMONAS AERUGINOSA* C1501 ON MONO AND DICO WEEDS

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Key words: weed management, submerged fermentation, carbon source, mineral salts, bio-herbicides.

Abstract

Microbe producing natural herbicides are alternatives to the chemical herbicidal formulations. The effect of minerals and carbon sources were screened to select the best when combined and when apply singly during submerged fermentation. The effect of their phytotoxic metabolites was tested on *Chromolaena odorata and Echinochola crus-galli.*

It was observed that the best combination between all the mineral was found in the combination containing manganese, zinc, bromine and iron. It gave the highest bio-herbicidal activities on the tested weeds when compared with the basal medium without any mineral amendment ($P \leq 0.05$). The best carbon source screened was glucose while the best mineral screened was iron in term of showing activities on the tested weeds ($P \leq 0.05$).

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WPŁYW ŚRODOWISKA HODOWLANEGO NA AKTYWNOŚĆ FITOTOKSYCZNĄ *PSEUDOMONAS AERUGINOSA* C1501 W ZWALCZANIU CHWASTÓW JEDNO- I DWULIŚCIENNYCH

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S ł o w a k l u c z o w e: gospodarka chwastami, fermentacja, źródło węgla, sole mineralne, bioherbicydy.

A b s t r a k t

Mikroorganizmy wytwarzające naturalne herbicydy są alternatywą dla chemicznych preparatów chwastobójczych. Badano wpływ soli mineralnych i źródeł węgla na wydajność fermentacji podczas osobnego i łącznego ich stosowania. Wpływ fitotoksyczności uzyskanych metabolitów testowano na *Chromolaena odorata* i *Echinochloa crus-galli*.

Wykazano, że spośród wszystkich badanych pierwiastków kombinacja zawierająca mangan, cynk, brom i żelazo dała najwyższą biologiczną aktywność chwastobójczą w porównaniu z podstawową pożywką bez zmiany składu mineralnego ($P \leq 0{,}05$). Pod względem aktywności fitotoksycznej najlepszym źródłem węgla była glukoza, a najlepszym pierwiastkiem żelazo ($P \leq 0{,}05$).

Introduction

Weeds are unwanted plants that compete directly and indirectly with economically important crops, reducing their yield, interfering with harvesting operations and provides hosts for insect pests and pathogens, as well as affecting the quality of the harvested product and thereby affecting the agricultural productivity of farmers in agricultural environments. This is one of the major reason mitigating against reduction in food production, in many parts of the world (BARRETO 2009, LORENZI 2000, ADETUNJI 2015).

The continuous usage of certain herbicides, or herbicides with similar mechanisms of action, in the same environment has led to the selection of weed populations that are resistant to certain chemical groups and becomes difficult to eradicate (OLIVEIRA JÚNIOR and INOUE 2011). Intensive and indiscriminate use of chemical pesticides might also lead to an ecosystem imbalance. This therefore necessitate the need for the use of biological agents for weed control particularly attractive (FONTES 1992, ADETUNJI and OLOKE 2013), following a global demand for an alternative control systems that are simultaneously effective, economic and less harmful to the environment.

It has been discovered that the active ingredient from most microorganisms are extracellular-secondary me-tabolites which are normally produced in culture me-dia serve as intermediates from primary metab-olisms as precursors for their biosynthetic process and they have various application as herbicides, anticancer agents, drugs, immunoregulators and antiparasitic agents. The environmental factors like as temperature and pH nutritional sources like carbon, nitro-gen, time, and minerals, have been discovered to have a profound influence on the activities of the active metabolite produced. Optimization of the culture conditions is essential to get high yields of the metabolites (SANCHEZ and DEMAIN 2002).

Therefore, this work intends to screen the best optimum mineral salt and carbon source when combine and apply singly that can influence the highest bio-herbicidal activities on the *Chromolaena odorata and Echinochola crus-galli* weeds.

Materials and Methods

Microorganisms and growth conditions

C1501 strain was isolated from the rhizosphere of wheat plants planted at the research farm of Nigerian Stored Product Research Institute, Ilorin Kwara State. The isolated bacteria was identified as *P. aeruginosa* C1501 with an accession number KF976394. The bacteria plates were incubated at 37°C for 48 h on Kings agar in BOD incubator. At the end of each incubation period, the colonies were subcultured onto fresh media maintained on slants of Kings agar and stored at 4°C in the refrigerator.

Optimization of *P. aeruginosa*

Bacteria were stored in 0.8% nutrient broth plus 0.5% yeast extract (NBY) broth (Difco, Detroit, Mich.) plus 40% glycerol at -80°C. Starter cultures were grown in 10-ml dilute (1/10-strength) NBY broth in 20-ml screw top vials for 8 to 12 h at 27°C at 140 rpm, yielding approximately 10^5 CFU ml^{-1}. Test cultures of 20 ml of NB or NBY broth (unbuffered) in 100-ml Erlenmeyer flasks were inoculated with 10 µl of starter culture. Chemical analysis indicated that NBY broth contained (mg l^{-1}): total nitrogen, 1441.0; amino nitrogen, 604.0; total phosphate, 600.1; potassium, 597.9; sodium, 259.7; chloride, 121.7; sulfate, 54.9; magnesium, 22.9; calcium, 6.1; zinc, 0.5; and boron, cobalt, copper, iron, lithium, manganese and molybdenum, < 0.1.

The sterile autoclaved medium was amended with filter-sterilized mineral solutions to give 1 mM $H^{33}O_3$, $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, LiCl, $MgSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $Mo_7(NH_4)_6O_{24} \cdot 4H_2O$, NaCl, 0.7 mM $CuSO_4$, $ZnSO_4 \cdot 7H_2O$, or 0.1 mM $CoCl_2 \cdot 6H_2O$ and with sterile stock solutions of carbon sources to give 1% (wt/vol). Cultures were incubated for 48 h at 24°C with shaking at 140 rpm in darkness, unless otherwise indicated. Culture pH for all media was 6.5 to 6.7 at inoculation and 7.7 to 7.9 after 48 h of bacterial growth. Bacterial growth after 48 h was approximately 10^8 CFU ml^{-1} in NBY.

In other experiments, combinations of the following minerals were investigated:

1. $ZnSO_4 + H_3BO_3 + FeSO_4 + MnCl_2$.
2. $ZnSO_4 + H_3BO_3 + FeSO_4 + (NH_4)_6Mo_7O_{24}$.
3. $ZnSO_4 + FeSO_4$.
4. $ZnSO_4 + FeSO_4 + (NH_4)_6Mo_7O_{24}$.

All cultures were provided with 134 μm EDTA as a chelating agent. Flask cultures were inoculated using 1 ml of a 24 h NBY adjusted to 10^6 CFU ml^{-1} in sterile distilled water. Cultures were incubated for 48 h at 130 rpm and 26°C in a rotary shaker incubator, thereafter cell density was measured at 600 nm and cultures were used for bioherbicidal assay. All glassware, including flasks, were acid-washed with 0.1% HCl solution to remove residual minerals. Their combined effect on the phytotoxic metabolite was later determined on sterilized leaves of monocotyledonous and dicotyledonous weeds. They were later transferred to Petri plate containing moistened cotton ball and filter paper. The sterilized leaves were then inoculated with cell free cultural filtrate containing 100 μl of the different combinations while NBY serves as control with the help of sterile needle on the surface of the leaf. Later, plates were incubated at 25°C for one week. A daily observation was made for the development of necrotic lesions from the inoculated leaves (SLININGER et al. 1996).

Effect of minerals and carbon source on the production of phytotoxic metabolites from *P. areuginosa*

P. areuginosa was grown for 48 h in 20 ml portions of nutrient broth yeast medium, 8 different NBY plus different carbon sources (maltose, glucose, sorbitol, mannitol, dulcitol, rhamnose, sucrose, glycerol) containing 1% (wt/vol) of the various media were screened so as to determine the best carbon source. The best carbon source was later combined with $FeSO_4$, $ZnSO_4$, H_3BO_3 and $(NH_4)_6Mo_7O_{24}$. Cultures were incubated for 48 h at 130 rpm and 26°C in a rotary shaker incubator, thereafter the cell density was measured at 600 nm.

The bioherbicidal assay was later carried out with the phytotoxic metabolite produced from the combination of the different minerals with the best carbon source on sterilized leaves of monocotyledon and diacotyledon weeds as described above (SLININGER et al. 1996).

Data analysis

The data were analyzed by using SAS software 8.2 (2001). Significant means were separated using Duncan's multiple range test.

The graphical scheme of experiments is shown in Figure 1.

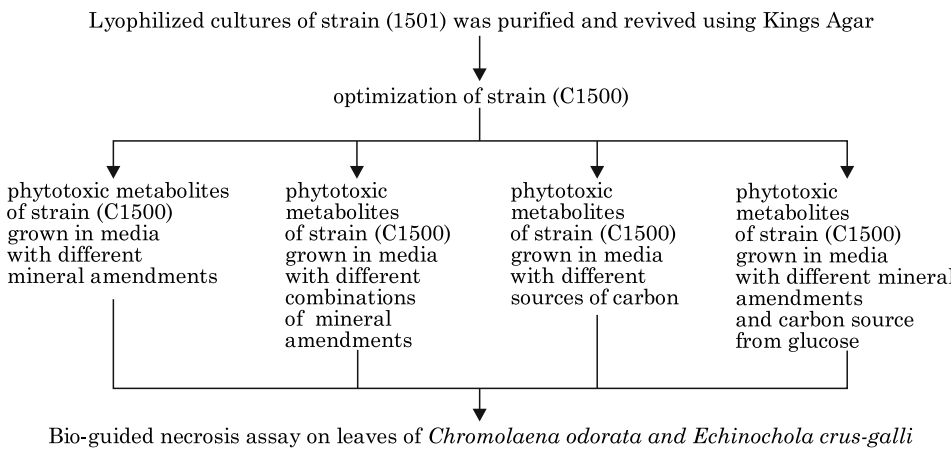

Fig. 1. Graphical scheme of experiments

Result

Optimization of *P. aeruginosa*

The phytotoxic activity of *P. aeruginosa* grown in media with different salt amendments was tested on *Chromolaena odorata and Echinochola crus-galli* leaves respectively. It was observed among all the tested minerals $FeSO_4$ amended with the basal medium produced the highest OD of 2.99 compared to the basal medium that had an OD of 1.63. The phytotoxic metabolite produced by *P. aeruginosa* induced a necrotic area of 3.5 mm^2 and 2.7 mm^2 ($P \leq 0.05$) compared to the basal medium without mineral amended that induced a necrotic area of 1.5 mm^2 and 1.0 mm^2 ($P \leq 0.05$) on *Chromolaena odorata and Echinochola crus-galli* leaves respectively (Table 1). It was observed that the

Table 1
Phytotoxic activity of *P. aeruginosa* grown in media with different mineral amendments on *Chromolaena odorata and Echinochola crus-galli*

Mineral amendments*	OD_{600}	Diameter of necrosis [mm]**	
		Chromolaena odorata	*Echinochola crus-galli*
Nutrient broth plus 0.5% yeast extract (NBY)	1.63 ± 0.7^{f}	0.9 ± 0.2^{e}	0.6 ± 0.21^{f}
Cacl	1.72 ± 0.31^{de}	1.5 ± 0.4^{de}	0.8 ± 0.1^{ef}
$MnCl_2$	2.95 ± 0.6^{a}	3.0 ± 0.6^{ab}	2.4 ± 0.3^{ab}
$CoCl_2$	1.78 ± 0.82^{d}	1.8 ± 0.2^{cde}	1.2 ± 0.5^{def}
H_3BO_3	2.31 ± 0.9^{c}	2.3 ± 0.25^{bcd}	1.9 ± 0.31^{abcd}
$FeSO_4$	2.99 ± 0.5^{a}	3.5 ± 0.7^{a}	2.7 ± 0.1^{a}
$ZnSO_4$	2.83 ± 0.4^{b}	2.8 ± 0.6^{abc}	2.1 ± 0.4^{abc}
$CuSO_4$	1.64 ± 0.9^{ef}	1.9 ± 0.2^{cde}	1.4 ± 0.6^{cdef}
$(NH_4)_6Mo_7O_{24}$	2.24 ± 0.52^{c}	2.0 ± 0.1^{bcde}	1.6 ± 0.4^{bcde}
LiCl	1.73 ± 0.7^{d}	1.7 ± 0.3^{cde}	0.9 ± 0.2^{ef}
NaCl	1.71 ± 0.6^{def}	1.6 ± 0.7^{de}	0.7 ± 0.1^{f}

Explanation: *medium without mineral amendments; **width of the diameter of necrosis on the eleaves. Means with different superscripts within the same column were not significantly different at 5%. Values are means ± standard error

best combination between all the mineral was found in the combination containing $ZnSO_4$ + H_3BO_3 + $FeSO_4$ + $MnCl_2$ with an OD of 3.21 compared to the basal medium without any mineral amendment with an OD of 1.93. The phytotoxic metabolite produced by *P. aeruginosa* induced a necrotic area of 3.8 mm^2 and 3.0 mm^2 ($P \leq 0.05$) compared to the basal medium without mineral amended that induced a necrotic area of 0.9 mm^2 and 0.6 mm^2 ($P \leq 0.05$) on *Chromolaena odorata and Echinochola crus-galli* leaves respectively (Table 2).

Different carbon sources were then screened to select the best carbon sources among all the tested sugars that produced the highest amount of metabolites. It was observed that when *P. aeruginosa* was inoculated into the different carbon sources, glucose produced the highest amount of phytotoxic metabolite with an OD of 3.0 while the basal medium without any mineral amendment with an OD of 0.7 (Figure 2*a*). The phytotoxic metabolite produced after the inoculation of *P. aeruginosa* into different carbon sources amended with different sugars showed that sucrose among all the sugars induced a necrotic area of 2.8 mm^2 and 2.4 mm^2 ($P \leq 0.05$) on *Chromolaena odorata and Echinochola crus-galli* leaves respectively (Figure 2*b*).

When the best carbon source screened was then combined with the best mineral salt, it was discovered that the combination containing $FeSO_4$ + glucose had an OD of 3.51 compared to the basal medium without any mineral amendment with an OD of 1.93. The phytotoxic metabolite produced from the medium that contained sucrose induced a necrotic area of 3.6 mm^2 and

Table 2

Phytotoxic activity of *P. aeruginosa* grown in media with different combinations of mineral amendments on *Chromolaena odorata and Echinochola crus-galli*

Mineral amendments*	OD_{600}	Diameter of necrosis [mm]**	
		Chromolaena odorata	*Echinochola crus-galli*
Nutrient broth plus 0.5% yeast extract (NBY)	1.93 ± 0.8^{b}	1.5 ± 0.4^{c}	1.0 ± 0.2^{b}
$ZnSO_4 + H_3BO_3 + FeSO_4 + MnCl_2$	3.21 ± 0.65^{a}	3.8 ± 0.5^{b}	3.0 ± 0.8^{a}
$ZnSO_4 + H_3BO_3 + FeSO_4 + (NH_4)_6Mo_7O_{24}$	3.19 ± 0.6^{a}	3.7 ± 0.2^{a}	2.9 ± 0.2^{a}
$ZnSO_4 + FeSO_4$	2.98 ± 0.7^{a}	3.6 ± 0.4^{ab}	2.7 ± 0.7^{a}
$ZnSO_4 + FeSO_4 + (NH_4)_6Mo_7O_{24}$	2.8 ± 0.4^{a}	2.5 ± 0.62^{bc}	2.1 ± 0.3^{ab}

Explanation: *medium without mineral amendments;**width of the diameter of necrosis on the leaves. Means with different superscripts within the same column were not significantly different at 5%. Values are means ± standard error

Table 3

Phytotoxic activity of *P. aeruginosa* grown in media with different mineral amendments and carbon source from glucose on *Chromolaena odorata and Echinochola crus-galli*

Mineral amendments*	OD_{600}	Diameter of necrosis [mm]**	
		Chromolaena odorata	*Echinochola crus-galli*
Nutrient broth plus 0.5% yeast extract (NBY)	1.93 ± 0.37^{d}	1.6 ± 0.2^{c}	0.9 ± 0.6^{d}
$FeSO_4$ + glucose	3.51 ± 0.83^{a}	3.6 ± 0.33^{a}	3.1 ± 0.2^{a}
$ZnSO_4$+ glucose	3.21 ± 0.4^{b}	3.21 ± 0.6^{ab}	2.53 ± 0.7^{b}
$(NH_4)_6Mo_7O_{24}$ + glucose	2.13 ± 0.6^{c}	2.83 ± 0.4^{ab}	2.10 ± 0.3^{c}
H_3BO_3 + glucose	2.02 ± 0.3^{cd}	2.42 ± 0.1^{b}	1.93 ± 0.5^{c}

Explanation: *medium without mineral amendments;**width of the diameter of necrosis on the leaves. Means with different superscripts within the same column were not significantly different at 5%. Values are means ± standard error

3.1 mm^2 ($P \leq 0.05$) on *Chromolaena odorata and Echinochola crus-galli* leaves respectively compared to basal medium that had 1.6 mm^2 and 0.9 mm^2 ($P \leq 0.05$) on *Chromolaena odorata and Echinochola crus-galli* leaves respectively (Table 3).

Discussion

Using microorganisms as a bioherbicide are uniquely capable of reducing invasive weed populations through highly specific impacts that are self-sustaining, contributing to the protection of natural ecosystems (DRIESCHE VAN et al. 2010). One group of microorganisms largely overlooked as biocontrol agents of weeds is the Deleterious Rhizobacteria (DRB) that can colonize plant root surfaces and able to suppress plant growth. A major group of rhizobacteria with potential for biological control is the *Pseudomonads*

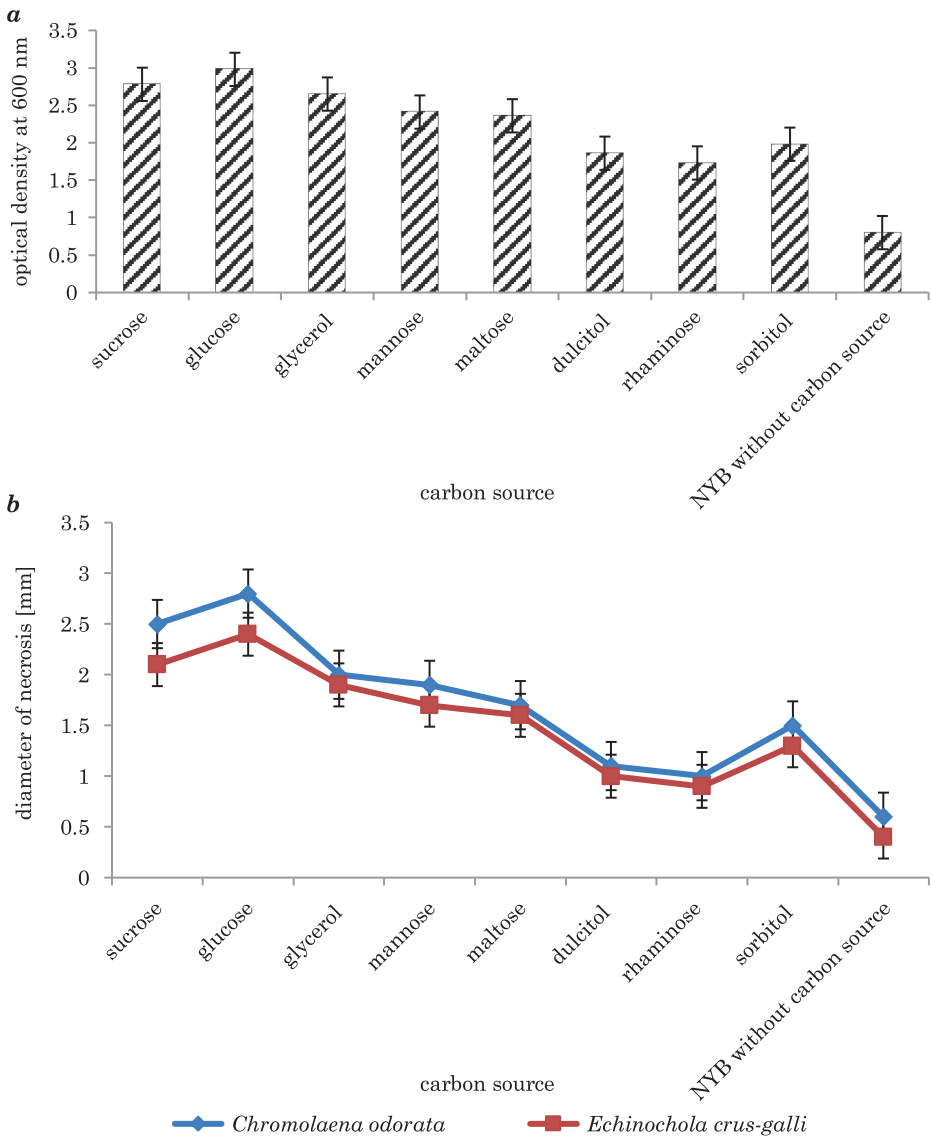

Fig. 2. Effect of: *a* – different sources of carbon on Optical density of P. *aeruginosa; b* – phytotoxic metabolites of *P. aeruginosa* produced from different sources of carbon on *Chromolaena odorata* and *Echinochola crus-galli*

(KENNEDY et al. 1991). During this study, *Pseudomonas aeruginosa* an example of the Deleterious Rhizobacteria (DRB) from Pseudomonades was used.

Optimization of media is generally done by studying the effects of the ingredients/nutrients on growth using fermentation studies, selecting and optimizing a few parameters (GRESHAM and INAMINE 1986). Nutritional factors such as carbon sources, nitrogen sources, trace metals, vitamins, carbon loading, and carbon-to-nitrogen ratio can all have an influence on growth, propagule formation, and biocontrol efficacy (JONSBU et al. 2002). Once an optimized defined medium has been developed, a production medium can be formulated by replacing the nutritional components of the defined medium with low-cost, complex substrates. Use of this directed optimization strategy not only aids in the development of production media for specific bioherbicides but also provides nutritional information which will be useful in developing production media for other microbial biocontrol agents. There are reports that medium and conditions used for production of biocontrol agents, influence their ability to survive during the formulation process (ZHANG et al. 2005). For example, mild thermal and pH stresses and carbon starvation can increase the resistance of cells to further stresses (OVERBEEK VAN et al. 1995).

Different mineral was amended with the basal medium in a submerged fermentation to screen out the best medium that produced the highest amount of phytotoxic metabolite on the tested weeds. It was observed that $FeSO_4$ and $ZnSO_4$ produced the highest colony forming unit and the largest necrotic area compared with the other mineral screened. Bacteria are usually mass-produced using liquid fermentation systems, but can also be produced through semisolid or solid-state fermentation (BOYETCHKO et al. 1999). Important conditions that must be considered are oxygen transfer, incubation temperature, nutrient requirement and agitation to ensure a large, stable, and efficacious bacterial population. The nutrients added to the medium should be inexpensive, readily available and conducive to a high biomass and proper secondary metabolite production (HYNES and BOYETCHKO 2006).

The combination of the basal medium with zinc in combination with molybdenum and iron improved the phytotoxic metabolite of *P. aeruginosa* after fermentation. There are some reports that zinc improves production of the antibiotics phenazine (OWNLEY et al. 2003, SLININGER and JACKSON 1992) and 2,4-diacetylphloroglucinol (DUFFY and DÉFAGO 1997, 1999).

It was observed that P. *aeruginosa* isolated from rhizospheres was able to produce a phytotoxic metabolite that can induce necrotic area on the leaves of tested weeds using submerge fermentation. KREMER et al. (1990) who reported that specific rhizobacteria which suppress weed growth are ubiquitous and probably found in all plant rhizospheres. Other examples of rhizosphere bacteria with bioherbicidal activity are *Enterobacter*, *Arthrobacter* and *Pseudomonas cichorii* (BOYETCHKO et al. 2002). This group of bacteria probably cause damage to the weed plant through the production of phytotoxins

that are taken up through the plant roots (KREMER et al. 1990). It has been reported that over 90% of fluorescent bacteria found in citrus root systems possess siderophore activity, which are likely involved in the suppression of weed growth (KREMER et al. 1990). In laboratory studies, over 100 bacterial isolates have been found to suppress the growth of grass weed roots by 80% (DAIGLE et al. 2002). According to KREMER et al. (1990), rhizobacteria will be successful in suppressing weed growth if they have a high colonizing ability, produce specific phytotoxin(s) that suppress growth of the host weed which are not suppressed by siderophores or antibiotics produced by competing microorganisms and have the ability to synthesize siderophores.

Green foxtail is another annual grassy weed, which is a weed of corn, soybean, cereals, canola, sugar beet, and pastures (DAIGLE et al. 2002). The suppression of weeds by *P. fluorescens* BRG100 has been attributed to secondary metabolites and phytotoxins. As mentioned previously, *Pseudomonas* spp. possess the ability to produce a variety of metabolites. This includes phytotoxins that cause symptoms such as root discolouration and reduced root length and also affect lipid synthesis and membrane integrity (BOYETCHKO et al. 2002).

It was observed that among all the carbon sources screened glucose followed by sucrose were able to produce the highest amount of colony forming unit and phytotoxic metabolites when *P. aeruginosa* was inoculated into the basal medium containing different carbon sources. Glucose is one of the primary molecules which serve as energy sources for almost all organisms, including bacteria. However, one of the most common growth media used in microbiology labs, nutrient broth, does not contain glucose as the main carbon source for bacteria (it contains protein). The addition of glucose to nutrient broth may increase the overall growth rates and biomass of bacteria over time. If so, this could be beneficial for lab purposes in that less time would be needed to grow cultures for experiments (NEIDHARDT et al. 1990). The result obtained during this study showed that glucose was a better carbon source is in line with NAMPOOTHIRI and PANDEY (1995) who showed that glucose gave best cell growth out of six carbon sources tested on *Brevibacterium* sp. in growth medium, EMANUILOVA and KAMBOUROVA (1992) who studied the effect of five carbon sources on *Bacillus steorothermophilus* found that the organism showed a preferential choice of growth and activity when grown on glucose as sole carbon source.

Conclusion

This study has shown that environmental factors had effect on the production of phytotoxic metabolite for the management of weeds from *P. aeruginosa* C1501 a potential bioherbicidal agent which could be an

alternative to the chemical herbicides. It was observed that when they were combined the highest bio-herbicidal activities was observed on the tested weeds when compared to the basal medium without any mineral amendment. Other studies to elucidate the active compounds still need to be carried out using from the metabolites, their mode of action, non-target effect and host range test of the active compound needs to be carried out as well as the greenhouse and field trial when compared with a chemical control. Therefore, the production of bioherbicides from this strain could be an alternative to the chemical herbicides which is not health and environmental friendly. We believe this work will be of great benefit and provide useful information for wide audience for so many sectors like for Industry, Environment and Agriculture. Also, it will provides vital information for entrepreneurs in business set up as well as farmers.

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OROBANCHE PALLIDIFLORA WIMM. ET GRABB. – SPECIMENS VARIABILITY AND PLANT COMMUNITIES – A CASE STUDY OF THE ABANDONED MEADOW

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K e y w o r d s: interindividual variability, parasitic plant, rare species, Western Pomerania.

A b s t r a c t

This paper presents the results of a research on the variability of *Orobanche pallidiflora* Wimm. et Grab. specimens which grew in an abandoned meadow in Lubiatowo (Pyrzyce County, West Pomeranian Voivodeship). Their spatial distribution was investigated and some biometric measurements were taken (shoot height, stem height, stem basal width, number of leaves, length of inflorescence, and number of flowers). Also participation of the species in plant communities was examined. 75 individuals of *Orobanche pallidiflora* were recorded in the sampling plot of 100 m^2. Their distribution was clustered. The majority of the biometric measures had low coefficient of variation values, except for the number of flowers (V = 59.63%). The specimens grew in species-poor *Molinietalia* communities of the Molinio-Arrhenatheretea class, where *Cirsium oleraceum* and *Cirsium arvense* were the dominant species.

OROBANCHE PALLIDIFLORA WIMM. ET GRABB. – ZMIENNOŚĆ OSOBNIKÓW I UDZIAŁ W ZBIOROWISKACH ROŚLINNYCH NA PRZYKŁADZIE NIEUŻYTKOWANEJ ŁĄKI

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Abstrakt

W artykule przedstawiono wyniki badań nad zmiennością osobników *Orobanche pallidiflora* Wimm. et Grab. występujących na nieużytkowanej łące w Lubiatowie (powiat pyrzycki, województwo zachodniopomorskie). W pracy badano ich rozmieszczenie przestrzenne, wybrane cechy biometryczne (wysokość pędu, długość łodygi, szerokość łodygi, liczbę łuskowatych liści, długość kwiatostanu i liczbę kwiatów) oraz udział gatunku w zbiorowiskach roślinnych. Na poletku badawczym o powierzchni 100 m^2 odnotowano 75 osobników *Orobanche pallidiflora* Wimm. et Grab. rozmieszczonych skupiskowo. Większość badanych cech biometrycznych osiągała niskie wartości współczynnika zmienności *V* – jedynie w przypadku liczby kwiatów wynosił on 59,63%. Osobniki badanego gatunku występowały w zbiorowiskach z rzędu *Molinietalia*, z klasy Molinio-Arrhenatheretea, o ubogim składzie florystycznym z przewagą *Cirsium oleraceum* i *Cirsium arvense*.

Introduction

Orobanche pallidiflora Wimm. et Grab. is a rare component of the Polish flora (PIWOWARCZYK et al. 2010). The species is considered to be endangered (EN) in Poland (KAŹMIERCZAKOWA et al. 2016), Western Pomerania (ŻUKOWSKI and JACKOWIAK 1995), Lower Silesia (KĄCKI et al. 2003), and the Sudets (FABISZEWSKI and KWIATKOWSKI 2002), as well as critically endangered (CR) in Gdańsk Pomerania (MARKOWSKI and BULIŃSKI 2004). It is currently under partial legal protection in Poland (Regulation of the Minister of Environment of 9th October 2014).

Orobanche pallidiflora is an annual, non-chlorophyllous, parasitic plant of a height of 70 (100) cm. It parasitises various species of the *Cirsium* and *Carduus* genera. It belongs to the Euro-Siberian sub-element – it ranges from France, Central and Southern Europe to the Ural Mountains and the Caucasus, and from Asia Minor to the Himalayas (MĄDALSKI 1967). The species has been reported from a few localities in Poland – mostly from Pomerania, Silesian Highland, and the Sudets (*Atlas rozmieszczenia*... 2001). After 2000, it has been confirmed at 51 localities in Poland: 7 localities in the Western Sudets (KWIATKOWSKI 2000, 2001, 2005, BACIECZKO and MYŚLIWY 2008), 37 in Western Pomerania (BACIECZKO 2002, BACIECZKO and MYŚLIWY 2005, 2008, PLUCIŃSKI and CHMIELEWSKI 2015), 5 in Belz Plain (PIWOWARCZYK et al. 2010), 2 in Middle Roztocze Region (PIWOWARCZYK et al. 2010), 2 in Romincka Forest (ŁACHACZ 2002), 1 in Volyn Polesia (PLUCIŃSKI and CHMIELEWSKI 2015), 1 in the Lower Vistula Valley (RUTKOWSKI unpbl., *Atlas rozmieszczenia*... 2001), 1 in Małopolska Upland (PIWOWARCZYK et al. 2010), and 1 in the Western Bieszczady Mountains (PIWOWARCZYK et al. 2010).

The paper is aimed at analysing the interindividual morphological attributes of *Orobanche pallidiflora* and investigating the floristic composition of plant communities accompanying the species.

Material and Methods

The specimens of *Orobanche pallidiflora* growing in an abandoned meadow situated in the vicinity of Lubiatowo village (53°09'37.5''N; 15°02'18.2''E; Pyrzyce County, West Pomeranian Voivodeship – Figure 1) were investigated. A field study was carried out in the growing season of 2015. A 100 m square sampling plot was established in a randomly chosen spot. The quadrat was than divided into 100 equal subplots of 1 m^2 each. The distribution of the specimens was mapped accordingly and it was used to evaluate the population size, specimens' density, and the population's type of spatial structure. Moreover, the Lloyd's index of mean crowding of specimens was determined (COLLIER et al. 1978) and the dispersion coefficient (*D*) was calculated according to TROJAN (1975). All *Orobanche pallidiflora* individuals were measured for the following morphological variables: 1 – shoot height, 2 – stem height, 3 – stem basal width, 4 – number of leaves, 5 – length of inflorescence, and

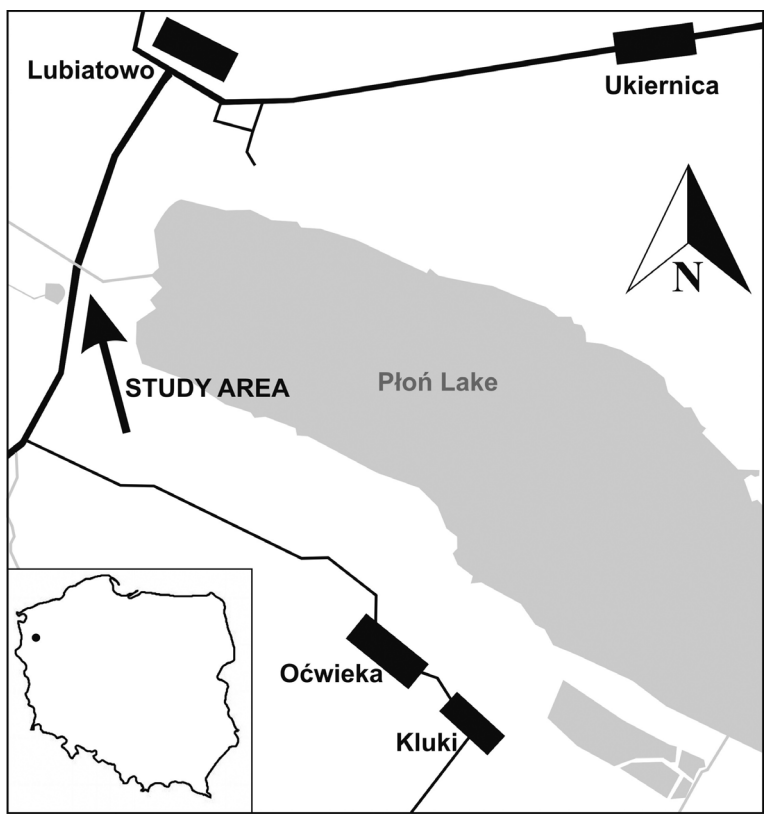

Fig. 1. Location of the study area

5 – number of flowers. Maximum, minimum, arithmetic mean, and coefficient of variation were calculated for each variable. Floristic composition of the plant communities with *Orobanche pallidiflora* was investigated using relevés conducted in 5 random patches located in the meadow. An extended Braun-Blanquet cover-abundance scale was used, adopted after DZWONKO (2007). The botanical names of vascular plants used in this paper follow MIREK et al. (2002), whereas the names of syntaxa were adopted after MATUSZKIEWICZ (2014).

Results

A total of 75 individuals were recorded within the 100 m^2 plot. The mean crowding was approximately 2 specimens per m^2. The population's density varied from 0 to 8 individuals per m^2 in different subplots. The value of the Lloyd's mean crowding index was 1.6 specimens. The cartographic data, as well as the value of the dispersion coefficient ($D > 1$) indicates the clustered type of spatial structure of the population (Figure 2).

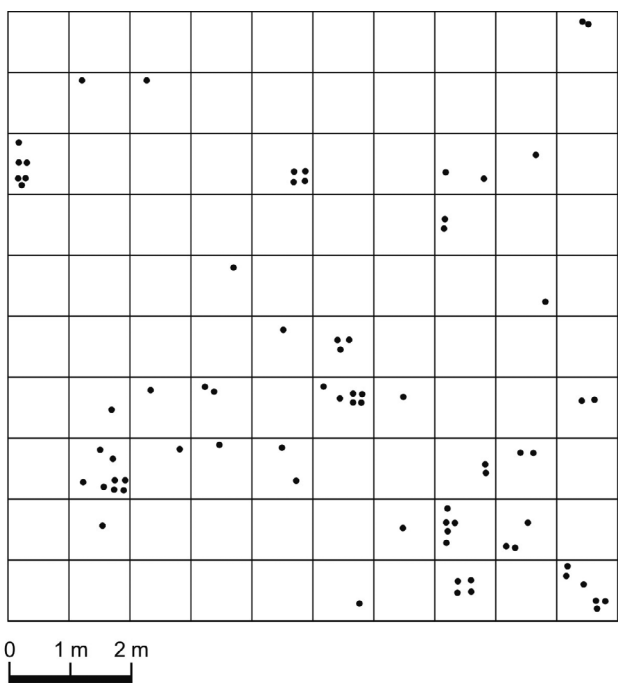

Fig. 2. Spatial structure of the *Orobanche pallidiflora* Wimm. & Grab. population investigated in Lubiatowo; • – individuals of *Orobanche pallidiflora* Wimm. & Grab.

Mean values of the morphological attributes are presented in Table 1. The shoots of *Orobanche pallidiflora* were 11–82 cm high and 1.3–16.6 mm wide (lower and taller specimens respectively). Shoots of the 61.8% of individuals were 21–40 cm high, whereas only 6 individuals developed shoots lower than 20 cm and 3 of them – shoots exceeding 60 cm. The value of coefficient of variation of the shoot height was low (V = 0.37%). The number of flowers was correlated to the length of inflorescence (r = 0.924545) – it varied between 0 and 65 flowers per individual.

Table 1

Main morphological traits of the individuals of *Orobanche pallidiflora* Wimm. & Grab. (n = 75)

Trait	x_{min}	x_{max}	$\bar{x}$	V[%]
Shoot height [cm]	11	82	35.6	0.37
Stem height [cm]	9	50	26.2	0.33
Stem basal width [mm]	1.3	16.6	8.1	0.34
Number of leaves	3	27	12.6	0.34
Length of inflorescence [cm]	2	32	9,4	0.59
Number of flowers	0	65	24.0	59.63

Explanations: x_{min} – minimum, x_{max} – maximum, $\bar{x}$ – arithmetic mean, V – coefficient of variation

The specimens of *Orobanche pallidiflora* occurred in species-poor meadow communities of the *Molinietalia* order, of the Molinio-Arrhenatheretea class. The relevé patches were dominated by *Cirsium oleraceum* and *Cirsium arvense*. Meadow species, e.g. *Galium mollugo* and *Deschampsia caespitosa* were also frequently recorded, as well as synanthropic species of the Artemisietea vulgaris class, e.g. *Eupatorium cannabinum*. Other common species that appeared in the patches were *Phalaris arundinacea* and *Symphytum officinale*. A detailed list of the species recorded at the study site is presented in Table 2.

Discussion

The *Orobanche pallidiflora* individuals mostly parasitised *Cirsium arvense*, whereas *Cirsium oleraceum* was a less frequent host, which is in line with the research of BACIECZKO and KLERA (2008), as well as with the study of BACIECZKO and MYŚLIWY (2008), who confirmed that in the Płonia Valley *Orobanche pallidiflora* parasitised the 2 species mentioned above. Also KWIATKOWSKI (2000, 2001, and 2005) and PIWOWARCZYK et al. (2010) reported the preference of the *Cirsium* genus hosts (*C. arvense*, *C. oleraceum*, *C. palustre,* and *C. vulgare*), along with hosts of the *Carduus* genus (*C. acantoides* and *C. personata*).

Table 2

Plant communities with *Orobanche pallidiflora* Wimm. & Grab.

Location	Lubiatowo					
Date	15.07.2015					
Area of relevé [m^2]	100					
Latitude [N]	53°09'36.0''	53°09'36.1''	53°09'36.3'	53°09'35.9''	53°09'35.8''	Constancy
Longitude [E]	15°02'19,4''	15°02'18.9''	15°02'18.9''	15°02'18.8''	15°02'18.4''	
Herbaceous layer cover [%]	100					
Number of species in the relevé	18	14	17	16	16	
Orobanche pallidiflora	2m1	2a1	2a2	2a2	2a2	V
Molinietalia						
Cirsium oleraceum	3.3	2b3	3.3	2a2	4.4	V
Deschampsia caespitosa	1.2	1.2	1.2	1.2	·	IV
Hypericum acutum	+	·	1.1	·	+	III
Lythrum salicaria	·	·	·	r	+	II
Molinio-Arrhenatheretea						
Galium mollugo	2m3	2m3	2m3	2a3	2a4	V
Potentilla anserina	+	·	+	+	2m1	IV
Juncus inflexus	1.2	·	1.1	·	1.2	III
Inula britannica	1.1	r	1.1	·	·	III
Achillea millefolium	·	+	+	·	·	II
Vicia cracca	·	·	·	+	+	II
Artemisietea vulgaris						
Cirsium arvense	2b3	3.3	2b3	3.4	2a3	V
Eupatorum cannabinum	2a3	2m1	1.1	2m1	1.1	V
Urtica dioica	+	+	·	+	·	III
Carduus crispus	+	·	·	+	·	II
Others						
Phalaris arundinacea	1.1	2b3	3.3	2a3	2m2	V
Symphytum officinale	2a2	2a2	2a2	2a2	2m2	V
Mentha arvensis	1.1	2m1	2m1	1.1	·	IV
Elymus repens	·	+	·	+	·	II
Mentha aquatica	·	·	+	·	+	II
Carex acutiformis	2a3					I

Sporadic: **ChO. Molinietalia:** *Stachys palustris* 2, **ChCl. Molinio-Arrhenatheretea:** *Plantago lanceolata* 5, *Dactylis glomerata* 5, *Phleum pratense* 5, **ChCl. Artemisietea vugaris**: *Linaria vulgaris* 3, **Others:** *Phragmites australis* 1, *Salix cinerea* 1, *Sonchus arvensis* 1, *Humulus lupulus* 3.

The investigated population covered an area of 620 m^2 and it comprised approximately 300 specimens in 2014, while in 2015 the number of individuals decreased to 150, which was still quite a lot comparing to other known

localities of the species. The studies of BACIECZKO and KLERA (2008), BACIECZKO and MYŚLIWY (2005, 2008), and PLUCIŃSKI and CHMIELEWSKI (2015) confirmed that the Płonia Valley was abundant in the species. However, the most numerous population in Poland, that exceeded 1000 specimens, was recorded in the Bieszczady Mountains by PIWOWARCZYK et al. (2010). Other localities of the species recorded in the Southern Poland comprised between a few (PIWOWARCZYK et al. 2010) and 300 individuals (ŁACHACZ 2002, KWIATKOWSKI 2001, 2005).

The highest number of specimens that simultaneously parasitised the same host individual, recorded during the research, was 6. BACIECZKO and KLERA (2008) in their study found 8 individuals feeding on one shared host plant.

The maximum height recorded in the sample of *Orobanche pallidiflora* individuals was 82 cm which is higher than the values reported by RUTKOWSKI (2007) and BACIECZKO and KLERA (2008) – 80 and 60 cm respectively. However, PLUCIŃSKI and CHMIELEWSKI (2015) found higher specimens (exceeding 100 cm) in West Pomeranian Voivodeship.

The *Orobanche pallidiflora* population occurred in the abandoned meadow which had been an alkaline fen in the past. According to PLUCIŃSKI and CHMIELEWSKI (2015) soils rich in calcium carbonate are beneficial to the species. In the west of Poland, *Orobanche pallidiflora* was recorded mostly in moist or wet meadows, in the vicinity of fish ponds or other small bodies of water, as reported by BACIECZKO and MYŚLIWY (2005). Also MĄDALSKI (1967) found the species in various types of grasslands and in shrublands. In Western Sudets it was recorded in limestone hills, in abandoned quarries and gravel pits (KWIATKOWSKI 2001). According to PIWOWARCZYK et al. (2010), the species occurred in anthropogenic habitats – along rocky roadsides and in built-up areas. BACIECZKO and KLERA (2008) recorded the most numerous *Orobanche pallidiflora* population in a fallow meadow (252 individuals) as opposed to the populations that grew in a fallow field and in an agricultural meadow (55 and 35 specimens respectively). This indicates that the species prefers meadows over other habitats. Only 10 individuals of the species were reported from segetal and ruderal habitats (PIWOWARCZYK et al. 2010).

Floristic composition of the communities with *Orobanche pallidiflora* was diversified. In the investigated patches the species grew in an abandoned meadow that formerly comprised assemblages of the Molinio-Arrhenatheretea class. The most abundant species recorded in the patches were: *Deschampsia caespitosa*, *Potentila anserina,* and *Gallium mollugo*, as well as some ruderal species (*Eupatorium canabinum* and *Urtica dioica*). According to the study of PLUCIŃSKI and CHMIELEWSKI (2015), *Orobanche pallidiflora* occurred in communities of *Molinion*, *Calthion,* and *Arrhenatherion elatioris* alliances. Moreover, depending on the type of habitat, the most abundant taxa reported

by PIWOWARCZYK et al. (2010) from the communities with *Orobanche pallidiflora,* were the characteristic species of the Molinio-Arrhenatheretea, Artemisietea vulgaris, Stellarietea mediae, Agropyretea intermedio-repentis, and Festuco-Brometea classes.

Conclusions

Considering the small number of localities of *Orobanche pallidiflora* in Poland, environmental monitoring of the species and its habitats is highly recommended. The site is valuable because it is abundant in individuals of the species. The study was a preliminary research and it will be continued in the future, particularly the spread of the species will be monitored.

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TEMPERATURE PRETREATMENT EFFECTS ON *TRIFOLIUM PRATENSE* L. SEED DORMANCY AND GERMINATION

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K e y w o r d s: hard seeds, hydrotime modelling, red clover, seed physiological parameters.

A b s t r a c t

The cause of seed dormancy relief may be various external factors, however the most data suggest particular role of temperature, especially it is seasonally changing environmental cue. The impact of temperature on hydrotime model parameters of red clover seeds has not been studied up to date. The aim of the study was to determine the water relations of red clover seeds during germination after different constant or fluctuating temperature pretreatment in a dry and moist seedbed, on the basis of the hydrotime model. The highest germination was obtained as a result of temperatures in a moist seedbed thanks to a shift of the mean base water potential towards negative values. Alternating positive temperatures broke the dormancy of red clover seeds to the greatest extent. The use of the hydrotime model to characterise and predict relief of combinational dormancy may be a very effective approach, especially for cultivars, which contains a small percentage of hard seeds. Red clover seeds do not need extreme temperatures or large amplitudes of temperatures alternation to break dormancy in temperate climates. Our results acknowledged the advisability of sowing red clover in autumn because exposition to winter and early spring conditions allow seeds to reach a high vigour and successfully emerge in spring.

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WPŁYW TEMPERATURY WSTĘPNEGO PRZECHOWYWANIA NA KIEŁKOWANIE I SPOCZYNEK NASION *TRIFOLIUM PRATENSE* L.

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S ł o w a k l u c z o w e: nasiona twarde, modelowanie hydroczasowe, koniczyna czerwona, parametry fizjologiczne nasion.

A b s t r a k t

Przyczyną ustępowania spoczynku fizycznego nasion mogą być różne czynniki zewnętrzne, jednak najwięcej danych sugeruje, że szczególną rolę może odgrywać temperatura, zwłaszcza że jest zmieniającym się sezonowo sygnałem środowiskowym. Dotychczas nie badano wpływu temperatury na parametry modelu hydroczasowego nasion koniczyny czerwonej.

Celem badań było określenie stosunków wodnych podczas kiełkowania nasion *Trifolium pretense,* wstępnie przechowywanych w stałych lub zmiennych temperaturach w suchych lub wilgotnych warunkach, w oparciu o model hydroczasowy. Najwyższe kiełkowanie otrzymano w wyniku działania różnych temperatur w środowisku wilgotnym, dzięki przesunięciu średniego progowego potencjału wodnego ku wartościom ujemnym. W największym zakresie spoczynek nasion koniczyny czerwonej został przełamany przez zmienne dodatnie temperatury. Zastosowanie modelu hydroczasowego do charakterystyki i przewidywania ustępowania mieszanego spoczynku okazało się bardzo obiecującym podejściem, szczególnie dla odmiany zawierającej niewielki procent nasion twardych. Nasiona koniczyny czerwonej do przełamania spoczynku w klimacie umiarkowanym nie wymagają ani ekstremalnych temperatur, ani zmiennych temperatur o bardzo dużej amplitudzie.

Wyniki badań potwierdziły celowość siewu koniczyny czerwonej jesienią, ponieważ ekspozycja na zimowe i wczesnowiosenne warunki pogodowe pozwala nasionom osiągnąć wysoki wigor i z sukcesem wschodzić wiosną.

Introduction

Trifolium pratense L. is a widely grown and important forage legume in many countries. However, its usefulness can be reduced due to low persistence. Physiologically, red clover is a perennial, but depending on field and weather conditions, it may behave like an annual, a biennial, or a short-lived perennial. For permanent use, seeds of this species have to be sown for several years. Although there are few studies on importance of red clover reproduction and seedling regeneration in this species perenniality. The data suggest that the red clover population can persist by natural reseeding in permanent meadows (SAKANOUE 2004).

The seed population of this species is usually comprised of a high proportion of hard seeds. Physical dormancy can be broken artificially by different treatments such as acid or mechanical scarification, or exposure to very high or very low temperatures (HERRANZ et al. 1998, MARTÍN, GUERRERO 2014, ŻUK-

-GOŁASZEWSKA et al. 2007). However, the softening of hard seeds in natural conditions is only partly understood. In many tropical and Mediterranean ecosystems, fire or very large daily temperature fluctuations are important factors (MORENO-CASOLA et al. 1994). Many authors suggest that passage through the animal's digestive tract, mechanical abrasion by soil particles or seed coat decomposition by microbial action are factors which cause hard seed permeability. However, direct evidence for it is lacking. Moreover, these factors are not dependent on the seasons of the year and many hardseed species from the *Fabaceae* family, such as red clover, exhibit seedling emergence seasonal pattern (BASKIN et al. 2000, ASSCHE VAN et al. 2003). Hard seeds need specific duration of time to become permeable and capable of germination. That is why many seeds that fall in late summer have to pass through winter before germination. There is some evidence that temperature can also be a factor in breaking the physical dormancy of the seeds of certain species in temperate climates, despite the fact that extreme temperatures do not occur there and temperature fluctuation amplitudes are not very large. It can be supposed that temperature acts as an environmental cue for the germination of hard seeds in temperate climates (ASSCHE VAN et al. 2003). It is known that in years with high temperatures and with low precipitation levels a red clover emergence is reduced (ŻUK-GOŁASZEWSKA et al. 2006).

The hydrotime model is a valuable approach to describing the phenomenon of seed germination in relation to available soil water and to analyse germination rates at different water potentials in a population. This model has explained the impact of different treatments on the seed germination of numerous species (BRADFORD 1990, BATLLA and BENECH-ARNOLD 2004, WANG et al. 2005, WINDAUER et al. 2012, BOCHENEK et al. 2009, 2010, 2016). BOCHENEK et al. (2007) have presented the influence of environmental conditions on field buried seed parameters derived from the hydrotime model. ŻUK-GOŁASZEWSKA et al. (2007) used this model to describe the physical dormancy break by acid scarification. The influence of different temperature pretreatment on red clover seed parameters derived from the hydrotime model has not yet been studied.

The aim of the study was to determine the water relations of red clover seeds during germination after different constant or fluctuating temperature pretreatment in a dry and moist seedbed, on the basis of the hydrotime model.

Material and Methods

Plant material

Red clover seeds (diploid cultivar Krynia) were obtained from field cultivation at the Experimental Station in Bałcyny, Poland (53°40'N, 19°50'E) and stored dry at room temperature (22–23°C) for one year. The initial germination was 70%, and after storage for one year, 73%. The mean value and the standard error of the mean of the calculated moisture content for all red clover seed samples after one year storage was 9.52% ± 0.14.

Seed treatment

After one year storage, four seed lots were packed separately in fine mesh nylon envelopes. Each envelope with seeds was buried in wet light loam (24% water) in a closed plastic pot and stored at constant temperatures of 3 or -10°C or fluctuating temperatures of -5/5, or 2/12°C, 12/12 h for 14 days. Other 4 seed portions were packed separately in paper bags and dry stored at the same temperatures for 14 days.

Germination test

Before the experiment and after storage of 14 days, the seeds representing each temperature and experimental variant were tested for germination at water and reduced water potentials (0, -0.2, -0.4, -0.6 and -0.8 MPa), which were determined utilizing polyethylene glycol (PEG 8000) solutions prepared as in MICHEL'S paper (1983). The water potentials were verified using a vapor pressure osmometer (Wescor model Vapro 5520) calibrated against NaCl standards. Germination tests were performed in glass 9 cm Petri dishes (3 replications of 50 seeds), on two layers of filter paper moistened with 5 ml of water or a PEG solution at the indicated ψ. The dishes were placed in plastic bags to prevent evaporation and were subsequently incubated in low-temperature incubators at a constant temperature of 19°C, for 5 days, except for brief periods when germination was scored. Seeds which did not germinate were transferred to fresh solutions after 48 h, to maintain a constant water potential in the dishes. Germination was recorded at 4, 12 or 24 h intervals depending on the rate of germination. The germination criterion was a visible radicle protrusion. Data were transformed into germination percentages and means were calculated.

Data analysis

The hydrotime model, initially proposed by GUMMERSON (1986) and developed by Bradford (1990), describes the relation between the germination rate of a given percentage g (GR_g) and the value of the difference between the seed water potential (ψ) and the physiological threshold water potential for germination of a given fraction g ($\psi_b(g)$). The form of the hydrotime model is:

$$\theta_H = (\psi - \psi_b(g)) \cdot t_g \quad (1)$$

$$GR_g = 1/t_g = (\psi - \psi_b(g))/\theta_H \quad (2)$$

where:
θ_H is the assumed hydrotime constant in seed population
t_g is the germination time for a specific fraction g.

The model supposes that ψ_b distinguishes among seed population fractions. The values of ψ_b are close to a normal distribution which can be characterized by its mean, $\psi_b(50)$ and standard deviation, σ_{ψ_b} (BRADFORD 2002).

Seed germination time courses in different water potential solutions were analyzed by probit regression according to the threshold population hydrotime model (Eqs 1, 2) and the computational procedure proposed by BRADFORD (2002) and GOŁASZEWSKI and BOCHENEK (2008):

$$probit_{(g)} = [\psi - (\theta_H/t_g) - \psi_b(50)]/\sigma_{\psi_b} \quad (3)$$

This procedure allowed the calculation of seed population hydrotime parameters, θ_H, $\psi_b(50)$ and σ_{ψ_b} and enabled the germination courses predicted for the model to be obtained. The coefficient of variation (CV) was expressed as a percentage of the mean.

Results

Red clover seed pretreatment in constant low positive and negative temperatures, both in wet and dry environments, caused an increase in final seed germination. The improvement of germination was slightly better for seed stored in humidity state (Table 1). Constant low temperatures caused a small increase of the $\psi_b(50)$ value (CV of 8% after wet storage and of 15% after dry storage) and decrease of the σ_{ψ_b} value (Table 1, Figure 1, Figure 2). The greatest changes were observed in the θ_H value, which distinctly decreased,

particularly in wet environments (CV of 61%). In most cases, the model fit well with the experimental data with values of R^2 from 0.74 to 0.84, so the parameters can be used to compare the effect of the treatment on seed germination performance (Table 1).

Table 1
Effect of constant temperatures on seed germination of red clover and hydrotime model parameters

Treatment	$\psi_b(50)$ [MPa]	σ_{ψ_b} [MPa]	θ_H [MPa h]	R^2	FG [%]
Before treatment	-0.423	0.396	20.1	0.809	72.7 ± 1.4
3°C, dry	-0.391	0.228	16.07	0.836	79.9 ± 4.9
-10°C, dry	-0.312	0.204	8.37	0.827	78.5 ± 4.6
Mean (CV)	-0.375 (15)	0.276 (36)	14.85 (38)	–	77.0 (5)
3°C,wet	-0.387	0.249	6.68	0.740	80.7 ± 4.6
-10°C, wet	-0.361	0.202	8.61	0.818	83.6 ± 4.9
Mean (CV)	-0.387 (8)	0.282 (36)	11.80 (61)	–	79.0 (7)

Explanation: CV – coefficient of variation expressed as a percentage of the mean; FG – final germination in water ± standard error; $\psi_b(50)$ – mean base water potential; σ_{ψ_b} – standard deviation of base water potential; θ_H – hydrotime constant; R^2 – coefficient of determination

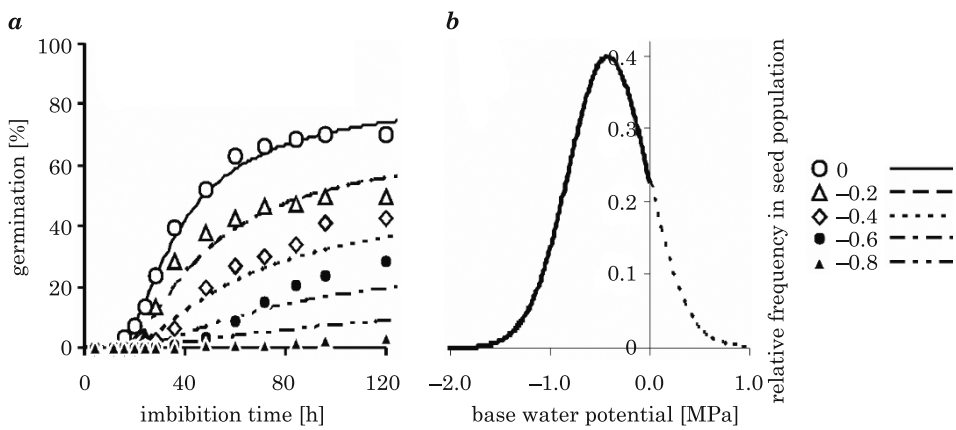

Fig. 1. Germination time courses of red clover seeds before experiment. The symbols are the actual data, and the lines are the time courses predicted by the hydrotime model using values shown in Table 1 and Table 2: *a* – germination time courses at 0(○), -0.2(△), -0.4(◊), -0.6(●) and -0.8(▲) MPa of seeds; *b* – normal distribution showing the relative frequencies of $\psi_b(g)$ values of seeds

Storage of *T. pratense* seeds for 14 days in alternating temperatures caused more variation in results. A seed treatment of alternating 12 h of -5°C and 12 h of 5°C in a dry environment caused a decrease in final germination. The same treatment in a moist environment produced a slight germination

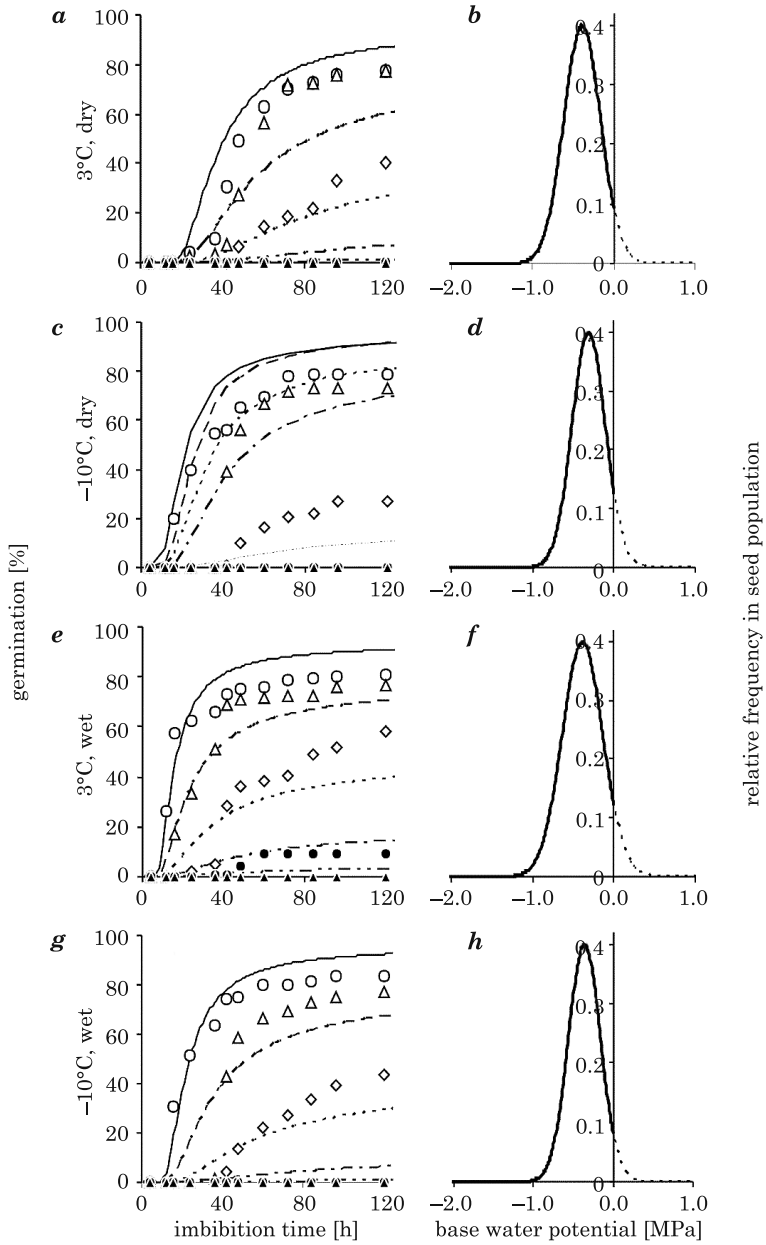

Fig. 2. Germination time courses of red clover seeds after constant low temperature pretreatment. The symbols are the actual data, and the lines are the time courses predicted by the hydrotime model using values shown in Table 1: *a, c, e, g* – germination time courses at 0(○), -0.2(△), -0.4(◊), -0.6(●) and -0.8(▲) MPa of seeds after storage at constant temperatures; *b, d, f, h* – normal distribution showing the relative frequencies of $\psi_b(g)$ values of seeds

increase (Table 2). Pretreatment in both environments brought about similar changes in hydrotime model parameters: a small shift to the right and also a small narrowing of the base water potential distribution (Figure 3). However, the hydrotime constant value was much lower in a wet than a dry seedbed (Table 1).

Table 2
Effect of fluctuating temperatures on seed germination of red clover and hydrotime model parameters

Treatment	$\psi_b(50)$ [MPa]	σ_{ψ_b} [MPa]	θ_H [MPa h]	R^2	FG [%]
Before treatment	-0.423	0.396	20.1	0.809	72.7 ± 1.4
-5/5°C, dry	-0.324	0.267	17.75	0.835	67.6 ± 3.4
2/12°C, dry	-0.318	0.169	7.71	0.859	87.9 ± 6.1
Mean (CV)	-0.355 (17)	0.277 (40)	15.19 (43)	–	76.1 (14)
-5/5°C, wet	-0.352	0.253	7.48	0.768	76.3 ± 2.2
2/12°C, wet	-0.627	0.440	18.28	0.640	90.8 ± 2
Mean (CV)	-0.467 (31)	0.363 (22)	15.29 (45)	–	79.9 (12)

Explanation: CV – coefficient of variation expressed as a percentage of the mean; FG – final germination in water ± standard error; $\psi_b(50)$ – mean base water potential; σ_{ψ_b} – standard deviation of base water potential; θ_H – hydrotime constant; R^2 – coefficient of determination

Alternating temperatures of 2/12°C distinctly increased final seed germination, although by several percent more in a wet than in a dry environment. Relatively high germination of dry stored seeds was connected with very low values of σ_{ψ_b} and θ_H, despite a less negative value of $\psi_b(50)$ than in the control sample. Wet stored seeds at 2/12°C germinated the best (90.8%), because a distinct shift of the mean base water potential value to the left, toward more negative values, although the distribution width of this parameter increased (Figure 3). The fit of experimental data for dry stored seeds at various temperatures was also good, with R^2 ranging from 0.84 to 0.90. The model fitted some worse with the experimental data for wet stored seeds with values of R^2 from 0.64 to 0.77 (Table 2).

Discussion

The occurrence of hard seeds in agricultural crop seed lots is considered undesirable because they contribute to non-uniform seedling emergence, potentially reducing yields, retarding harvest and diminishing the ability to compete with weeds. However, hardseededness may be regarded as desirable in certain situations, such as strong winter conditions, extended drought,

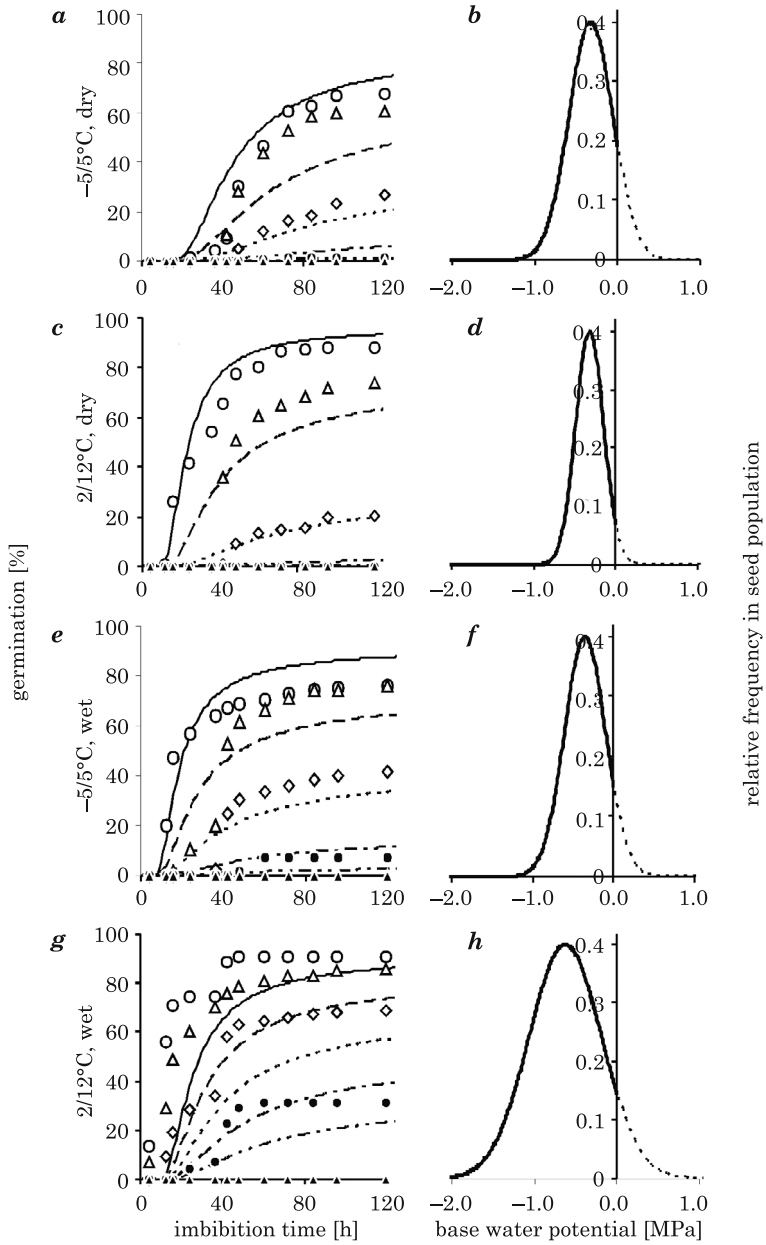

Fig. 3. Germination time courses of red clover seeds after alternating low temperature pretreatment. The symbols are the actual data, and the lines are the time courses predicted by the hydrotime model using values shown in Table 2: *a, c, e, g* – germination time courses at 0(○), -0.2(△), -0.4(◊), -0.6(●) and -0.8(▲) MPa of seeds after storage at fluctuating temperatures; *b, d, f, h* – normal distribution showing the relative frequencies of $\psi_b(g)$ values of seeds

or animal digestive tracts, where this feature permits a part of the seed population to survive (DEGREEF et al. 2002, ASSCHE VAN et al. 2003).

The hydrotime model fitted our experimental data quite well, so the parameters can be used to compare the effect of the treatment on seed germination performance. The values of R^2 showed that the model worked well to characterize germination time courses of red clover seeds at reduced water potentials and was comparable with the values obtained for other seed species (BATLLA and BENECH-ARNOLD 2004, HUARTE and BENECH-ARNOLD 2005, BOCHENEK et al. 2010, 2016).

The analysis of variation of hydrotime constant value was particularly significant in the case of seeds in physical or combinational dormancy. The decrease of θ_H value indicates germination acceleration of the examined seed population, which is connected with shortening of germination phase II in seeds with physiological dormancy (BRADFORD 2002). The dormancy mechanism is completely different in hard seeds. They cannot germinate, as the impermeable seed coat does not make water uptake possible. Softening the seed coat by scarification enables imbibition. If there are more seeds with damaged testae in a population, it germinates more rapidly and synchronically, because the hydrotime constant value is diminished (ŻUK-GOŁASZEWSKA et al. 2007).

The considerable decrease θ_H value after seed treatment by constant low temperatures suggested that such conditions, especially in a wet environment, caused an increase in the water permeability of the testa. Simultaneously, a small increase in germination percentage was connected with decreased vigour and sensitivity to reduced water potential (an increase in mean base water potential value). Seeds stored in fluctuating temperatures (5/-5°C) behaved similarly.

Alternating positive temperatures had a stimulating effect on red clover seed germination. The increase in germination percentage in dry conditions was connected with increased damage to the seed coat (a decrease in hydrotime constant value). There is large probability that the dry alternating temperature treatment resulted in the breaking or opening of specified structures in the testa e.g. lens or hilar fissure (HU et al. 2009). In the Krynia cultivar of clover seeds treated with concentrated sulphuric acid, the effect of which is mainly to increase seed coat permeability, the hydrotime constant value reduced most of all (ŻUK-GOŁASZEWSKA et al. 2007).

In alternating, low, above zero temperatures in a wet environment the water permeability of testae probably increased to a lesser extent; these conditions could also affect the physiological component of seed dormancy connected with the base water potential value (BASKIN and BASKIN 2004, FINCH-SAVAGE and LEUBNER-METZGER 2006). However, in such conditions this

physiological element of dormancy did not decrease uniformly throughout the entire seed population (giving a high value for σ_{ψ_b}). It was the reason for incomplete germination, which other authors also observed (RIDAY 2008). A similar pattern of hydrotime model parameter variations has been observed in several non-cultivated species whose seeds are characterized by physiological dormancy (HUARTE and BENECH-ARNOLD 2005, BATLLA and BENECH-ARNOLD 2004, HU et al. 2013). ALVARADO and BRADFORD (2005) suggest that if the processes mimic one another in terms of the pattern of variations in hydrotime model parameters, their mechanisms could also be similar. However, there is no doubt that alternating positive temperatures broke the combinational dormancy (P+Y) of red clover seeds to the greatest extent (BASKIN and BASKIN 2004, FINCH-SAVAGE and LEUBNER-METZGER 2006).

Temperatures, especially fluctuating temperatures, could be an environmental cues partly responsible for breaking the combinational dormancy of red clover seeds. Our results confirmed earlier data showing that red clover seed dormancy may be broken in relatively low temperatures leading to consistently better seedling emergence (ASSCHE VAN et al. 2003, ŻUK-GOŁASZEWSKA et al. 2006). Our results acknowledged the advisability of sowing red clover in autumn. The seeds that plants shed over a given area in late summer or autumn, are then exposed to winter and early spring conditions, which allow them to reach a high vigour and successfully emerge in spring. We have confirmed that seeds do not need extreme temperatures or large amplitudes of temperatures alternation to break combinational dormancy in temperate climates. However, such conditions cause partial distribution of germination over time, as a certain proportion of seeds remain dormant and there is possibility that they will germinate in the next autumn or after the next winter. From point of view of species regeneration, this is a beneficial effect because it is more probable that part of the seedling population will survive and the next generation will give new seeds, unlike a case in which all the population germinates uniformly.

Conclusions

1. Fluctuating positive temperatures in a wet seedbed broke the combinational dormancy of red clover seeds to the greatest extent.

2. *T. pretense* seeds do not need alternating temperatures of large amplitudes or extreme temperatures to break dormancy in temperate climates.

3. The hydrotime model turned out to be a very effective approach to characterize and predict relief of combinational dormancy.

4. Red clover sowing in autumn is advisable because exposition to winter and early spring conditions allow seeds to reach high vigour and successfully emerge in spring.

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CARPOLOGICAL MATERIAL AS AN INDICATOR FEN PEATLANDS DEGRADATION

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K e y w o r d s: degraded fen peatland ecosystems, plant macroremains, fruits and seeds of non peat-forming plant species, western Poland.

A b s t r a c t

The abandonment of agricultural use of drained fen peatlands contributes to intensified moorshing process and, consequently, faster of wetlands areas degradation. Peat mineralization causes the eutrophication of the habitat which, along with changing humidity, enables the presence of so-called non peat-forming plants. Among the seeds and fruit present in the top layer of degraded marshy soils, there may also be carpological material originating from non peat-forming plants. 12 soil profiles from selected post-marsh meadows in western Poland were the subject of this study. The studies found seeds and fruit of 69 plant species in moorsh layers, 42 of which were peat-forming species but as many as 27 were non peat-forming plants. Of the latter the most common were, among others, *Juncus effusus* and *Juncus conglomeratus*, *Urtica dioica,* which were characterized by the highest quantitativeness of fruit and seeds. Among the recorded non peat-forming species almost 50% belonged to *Molinio-Arrhenatheretea* class, which means they were species connected with semi-natural and anthropogenic meadows occurring on mineral or organic-mineral soils but also characteristic of muck emerging from degraded fen peatlands. Peat-forming species belonged to *Phragmitetea* and *Scheuchzerio-Caricetea nigrae* classes. Studies showed that the set of fruit and seeds of non peat-forming plants found in the upper layers of peat deposit was not dependent on the thickness or type of peat from which the moorsh originated, nor was it dependent on geographical location.

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MATERIAŁ KARPOLOGICZNY JAKO WSKAŹNIK PROCESU DEGRADACJI TORFOWISK NISKICH

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S ł o w a k l u c z o w e: zdegradowane torfowiska niskie, makroszczątki roślinne, owoce i nasiona nietorfotwórczych gatunków roślin, zachodnia Polska.

A b s t r a k t

Zaprzestanie użytkowania rolniczego odwodnionych torfowisk niskich przyczynia się do zwiększenia intensywności procesu murszenia torfu, a tym samym szybszej degradacji siedlisk bagiennych. Mineralizacja torfu powoduje eutrofizację siedliska, co wraz ze zmianą stopnia uwilgotnienia umożliwia wkraczanie tzw. nietorfotwórczych gatunków roślin. Wśród nasion i owoców znajdujących się w stropowej warstwie zdegradowanej gleby bagiennej może pojawiać się również materiał karpologiczny pochodzący od gatunków nietorfotwórczych. Przedmiotem badań było 12 profili torfowych pochodzących z wybranych łąk pobagiennych znajdujących się w zachodniej Polsce. W trakcie analiz w warstwach murszu rozpoznano nasiona i owoce 69 gatunków roślin, z czego 42 stanowiły gatunki roślin torfotwórczych, a aż 27 – gatunki roślin nietorfotwórczych. Najczęściej odnotowywanymi gatunkami nietorfotwórczymi były m.in.: *Juncus effusus* i *Juncus conglomeratus* oraz *Urtica dioica,* które charakteryzowały się jednoczenie największą liczebnością owoców i nasion. Wśród rozpoznanych gatunków nietorfotwórczych prawie 50% stanowili przedstawiciele klasy *Molinio-Arrhenatheretea*, czyli gatunki związane z półnaturalnymi i antropogenicznymi zbiorowiskami łąkowymi występującymi na glebach mineralnych lub organiczno-mineralnych i charakterystycznych także dla murszy wytworzonych z torfu niskiego. Gatunki torfotwórcze były zaś reprezentowane głównie przez przedstawicieli klasy *Phragmitetea* i *Scheuchzerio-Caricetea nigrae*.

W badaniach wykazano, że zestaw nasion i owoców gatunków roślin nietorfotwórczych, odnalezionych w wierzchnich warstwach złóż torfowych, nie był zależny od miąższości warstwy murszu oraz rodzaju torfu, z którego powstał mursz, jak również od położenia geograficznego obiektu.

Introduction

Wetland and marshy meadows play a significant role in the rural landscape of Poland, even though they only constitute 10% of hydrogenic habitats (DEMBEK 2002). They are, however, and similarly to active peatlands, endangered by human activity. Agricultural use of peatlands caused the drying out of hydrogenic habitats. Political and economic changes in Poland at the end of the XX century led to the abandonment of many wetland meadows. It is now known that the abandonment of drained wetland meadows leads to intense degradation of peat soil through ever deeper moorhing process (BRANDYK et al. 2007, DEMBEKA and PIÓRKOWSKI 2007, ILNICKI and SZAJDAK 2016). As a result, the thickness of peat decreases and, eventually, disappears. Consequently, on

over 80% of Polish peatlands the peat-forming process disappear (GROOTJANS and WOŁEJKO 2007). The proceeding mineralization of peat changes habitat conditions, which makes it possible for non peat-forming plant species to occur (KRYSZAK et al. 2004, KUCHARSKI 2008, TOMASZEWSKA et al. 2011).

A proper evaluation of the dangers to peatlands is only possible when it is based on complex studies of particular sites (DOBROWOLSKI et. al. 1998). A set of research and diagnostic tools of wetlands needs to be used. From the botanical point of view, the study needs not only the characteristics of plants presently occurring in the peatlands but also a historical approach to their development. The stratigraphic analysis is one of the most important paleobotanic methods revealing the genesis of a peatland. Plant macroremains analysis is divided into two parts: reproductive (seeds and fruit) and vegetative structures. Seeds and plants in peat soil form a rich seed-bank, with regard to both the degraded layer and the unchanged peat deposit. Therefore, it could be assumed that floristic changes occurring on marshy meadow surface should also be reflected in the macroremains composition occurring in the mineralized fen layer, namely the moorsh. Consequently, it seems more prudent to analyze peat deposits including both the unchanged and the degraded part (KLIMKOWSKA 2006, KOŁODZIEJCZYK and TOMASZEWSKA 2010). The depth of non peat-forming seed and fruit occurrence could indicate the thickness of the degraded peat soil – moorshing process.

The aim of the research is to establish a list of indicator plant species whose carpological material (seeds and fruit) found in the upper layers of peat deposits could inform us about the steps of the mineralization (moorshing process) of peatlands and, at the same time, about its degradation.

Materials and Methods

Seven meadows located on fen peatlands in Poland were selected for the study (Table 1). The sites were selected using the following criteria:

– post-marsh meadows on fen peatlands used for agriculture in the past or unused for over a decade;

– the thickness of peat layer should exceed 1m, as both the degraded and the unchanged part of the deposits were to be studied;

– the studied sites should be located in various parts of western Poland.

12 soil profiles were collected using an Instorf type drill with canister diameter of 5 cm and height of 50 cm. The material for studies was both moorsh in the upper part of the peat profiles and peat occurring in lower parts. In the laboratory the collected profiles were divided into 5 cm parts (around 20 cm^3 of volume), which were then samples for stratigraphic analyses. The paleobotanic research included the following:

Table 1

Study sites – location and usage

Name of peatland	Voivodeship	Name of profiles	Geographical location	Usage
Miękinia	Lower Silesian Voivodeship	Miękinia	N51°11'59.4'', E16°43'19.24''	secondary paludification
Koskowice	Lower Silesian Voivodeship	Koskowice	N51°11'3.33'', E16°15'13.75''	unused meadow
Milicz	Lower Silesian Voivodeship	Milicz 1	N51°35'43.17'', E17°20'24.1''	used meadow
		Milicz 2	N 51°34'59.56'', E 17°12'26.06''	unused meadow
		Milicz 3	N51°34'59.08'', E17°12'25.58''	unused meadow
Borek	Opole Voivodeship	Borek 1	N51°08'38.6'', E18°16'55.0''	unused grassland
		Borek 2	N51°08'41.3'', E18°16'53.3''	unused meadow
Czarnocin	West Pomeranian Voivodeship	Czarnocin	N53°45'10.8'', E14°33'57.5''	unused meadow
Widzieńsko	West Pomeranian Voivodeship	Widzieńsko	N53°39'50.4'', E14°46'58.9''	unused meadow
Kikorze	West Pomeranian Voivodeship	Kikorze 1	N53°37'53.4'', E15°00'08.1''	pasture
		Kikorze 2	N53°37'51.4'', E15°00'07.8''	unused meadow
		Kikorze 3	N53°37'51.95'', E15°0'6.97''	unused meadow

1. determining the ash content in peat through burning 1/5 of each section in a muffle furnace at 600°C for 4 hours – ash content was determined in accordance with Polish Norm (*Torf i wyroby*... PN-G-04596).

2. determining the botanical content of peat – the samples were placed in 10% NaOH solution and later rinsed under water on a sieve with 0,3 mm meshes. The rinsed plant remains were segregated under a stereoscope microscope into vegetative structures and seeds/fruit. Carpological material was of particular significance. Atlases and other sources were used to identify the remains, among others the following: BERGGREN (1969), KATZ et al. (1965, 1977), GROSSE-BRAUCKMANN (1972, 1974) GROSSE-BRAUCKMANN, STREITZ (1992), CAPPERS et al. (2012).

The types and species of peat were determined on the basis of botanical plant remains in peat in accordance with the system of genetic classification (TOŁPA et al. 1967, PARENT and ILNICKI 2002) and the Polish Norm (*Torf. Genetyczny*... PN-85/G-02500).

Plant species names were taken from MIREK et al. (2002). Classifying particular species into particular phytosociological classes was based on the studies by MATUSZKIEWICZ (2002).

Results

In each of the studied peatlands there was a clearly visible layer with initiated moorshing process. Miękinia location was an exception – the whole profile with thickness of only 0.3 m was made of moorsh. In other profiles the degrading marshy soil had thickness between 0.1 m (Widzieńsko, Kikorze 1 and Kikorze 3) to 0.27 m (Milicz 2), while 0.2 m was the most common result (Table 2). These thicknesses were consistent with the extent of levels determined on the basis of higher ash content.

The analyses of moorsh layers in the profiles revealed seeds and fruit of 69 plant species, 42 of which were peat-forming plants (belonging to classes *Scheuchzerio-Caricetea nigrae* and *Phragmitetea* and occurring in communities characteristic of fen peatlands) and as many as 27 non peat-forming species occurring only in dried out and degraded peatlands (Table 3). The analyses also showed fruit fragments impossible to fully identify, belonging to *Potentilla* genus and *Asteraceae* family.

Table 2

Peat species occurring under the moorsh layer along with the number of species indicating the moorshing process and the number of peat-forming species found in muck layers of the studied profiles

Profile	Moorsh layer [m]	Peat species under the moorsh layer	Number of species indicating the moorshing process	Number of peat-forming species in moorsh layer
Miękinia	0.30	only moorsh	**1**	9
Koskowice	0.20	sedge peat	**7**	7
Milicz 1	0.14	sedge peat	**7**	8
Milicz 2	0.27	sedge peat	**7**	11
Milicz 3	0.20	sedge peat	**11**	16
Borek 1	0.20	sedge peat	**3**	6
Borek 2	0.20	sedge-moos peat	**2**	6
Czarnocin	0.20	sedge peat	**2**	6
Widzieńsko	0.10	sedge peat	**3**	8
Kikorze 1	0.10	*Drepanocladus* moos *(Drepanocladus)* peat	**5**	4
Kikorze 2	0.15	*Drepanocladus* moos *(Drepanocladus)* peat	**7**	10
Kikorze 3	0.10	*Drepanocladus* moos *(Drepanocladus)* peat	**4**	6

Table 3
The index of plants indicating the decaying process in peat along with the number of occurrences of a given species and the number of seeds/fruits in the moorsh layers of the studied peat profiles

Species	Phytosociological classes	Number of occurrences in 12 profiles	Number of seeds/fruit in moorsh layers
Agrostis gigantea Roth	Mol.-Arrh.	1	5
Alopecurus pratensis L.	Mol.-Arrh.	1	8
Anthoxanthum odoratum L.	Koel	1	70
*Calamagrostis epigejos (*L.) Roth	Epilob. ang.	1	1
Cisrium palustre (L.) Scop.	Mol.-Arrh.	2	1–3
Geranium palustre L.	Mol.-Arrh.	2	1–9
Holcus lanatus L.	Mol.-Arrh.	1	1
Hydrocotyle vulgaris L.	Scheu.-Car. nigrae	4	1–15
Juncus conglomeratus L. Em. Leers	Mol.-Arrh.	5	54–853
Juncus effusus L.	Mol.-Arrh.	7	1–699
Mentha arvensis L.	-	3	4–5
Moehringia trinervia (L.) Claivr.	Atrem.	1	1
Molinia caerulea (L.) Moench.	Mol.-Arrh.	1	8
Polygonum bistorta L.	Mol.-Arrh.	1	2
Polygonum lapathifolium L.	Bid. trip.	1	1
Potentilla anserina L.	Mol.-Arrh.	3	1–7
Potentilla argentea L.	Koel.	2	7–22
Potentilla erecta L.	Nardo-Callun.	2	2–15
Potentilla sp.	–	2	1–2
Ranunculus acris L.	Mol.-Arrh.	4	2–18
Ranunculus repens L.	Mol.-Arrh.	6	1–25
Ranunculus sceleratus L.	Bid. trip.	4	1–21
Stellaria holostea L.	Quer.-Fag.	1	5
Trifolium repens L.	Mol.-Arrh.	3	1–4
Urtica dioica L.	Artem.	6	1–137
Viola arvensis Murray	Stell. med.	3	1
Others from *Asteraceae*	–	3	1

Class names: Mol.-Arrh. – *Molinio-Arrhenatheretea*, Epilob. ang. – *Epilobietea angustifolii*, Scheu-Car. nigrae – *Scheuchzerio-Caricetea nigrae*, Atrem – *Artemisietea*, Bid. trip. – *Bidentetea tripartiti*, Nardo-Callun – *Nardo-Callunetea*, Quer.-Fag. – *Querco-Fagetea,* Stell. med. – *Stellarietea mediae,* Koel. – *Koelerio glaucae-Corynephoretea canescentis.*

The fruit and seeds most clearly indicating the moorshing process belong to *Juncus conglomeratus* and *Juncus effusus* (recorded in 12 profiles) and *Urtica dioica* (occurred 6 times). The number of sporomorphs of these species in the moorsh layers was varied, but sometimes very high (Table 3). For instance,

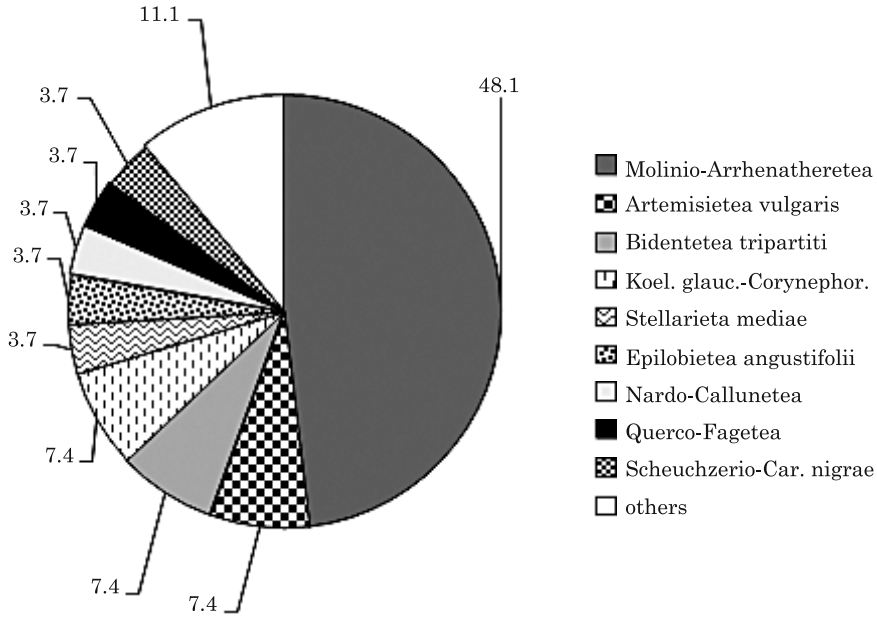

Fig. 1. The percentage of phytosociological classes of non peat-forming seeds and fruits plant species recorded in the degraded layers of all analyzed peat profiles

the profile Borek 1 had 137 fruits of *Urtica dioica*, whereas the profile Milicz 3 had 853 seeds of *Juncus conglomeratus* (Table 3) The carpological material of other non peat-forming plant species occurred less often, e. g. *Potentilla anserina* was recorded 3 times, *Ranunculus sceleratus* and *R. acris* – 4 times, and *Polygonum bistorta* only once. In profiles Kikorze 2, Milicz 1, Milicz 2 and Milicz 3 *Hydrocotyle vulgaris* was recorded. The species does belong to the *Scheuchzerio-Caricetea nigrae* class, but in Polish fen peatlands it is a sign about the initiation of peat drying out in the upper layers of peat deposit. The recorded seeds and fruit may also indicate the earlier was fen peatlands were used. Consequently, seeds of *Trifolium repens* (Koskowice, Milicz 2, Milicz 3) and fruit of *Alopecurus pratensis* (Borek 2)*, Anthoxanthum odoratum* (Kikorze 2) and *Holcus lanatus* (Milicz 3) confirm previous peatland usage as meadows. In all profiles, in the moorsh layer, the study recorded a mosaic of seeds and fruit of peat-forming and non peat-forming species. However, in each location there was a different combination and a different number of species of both groups (Table 3). Research suggests that geographical location and peat type from which moorsh originates do not influence the composition or number of non peat-forming species. (Table 2, Table 3). Similarly, on the amount of seeds and fruit of particular plant species.

Non peat-forming plant species, whose seeds and fruit were noted in moorsh layers belong to 9 phytosociological classes (Figure 1).

Molinio-Arrhenatheretea class was the most numerous with 13 species (48,1% of 27 species). Species belonging to this class are connected with semi-natural and anthropogenic grassland communities occurring on mineral or organic-mineral soils and also characteristic of moorsh originating from fen peatlands (MATUSZKIEWICZ 2002, DIERSSEN K. and DIERSSEN B. 2008). The other classes are presented to a lesser extent (Figure 1).

The research suggests that all 27 species (Table 3) can be seen as indicative of peat decay process.

Discussion

The obtained results are confirmed by previous studies. While describing Rabinówka peatland, DRZYMULSKA (2004) recorded the presence of *Urtica dioica*, *Ranunculus acris* and several fruit of species from *Asteraceae* family in the decaying surface layer of the peat deposit. Similarly, the peatland near Pasikurowice (Lower Silesia) had seeds of *Juncus effusus* and fruits of *Urtica dioica* in the upper layer of decaying peat (MALKIEWICZ et al. 2015), and the latter was more numerous – 31 fruits. In Równina Wełtyńska (north-western Poland, near Czarnowo) there is a peatland with visible degenaration of peat-forming communities. The changes are a reflection of an ongoing moorshing process. The upper 0,1m had seeds of *Juncus effusus* and fruits of *Ranunculus acris* (MALKIEWICZ and TOMASZEWSKA 2009) An even higher number of non peat-forming species was recorded in upper layers of Większyce peatland (Opole Voivodeship) (TOMASZEWSKA 2012) with seeds/fruit of *Urtica dioica, Ranunculus repens, Stellaria holostea, Juncus effusus* and *Trifolium repens.* The presence of *Triforium repens* suggests previous usage of the peatland as a meadow.

Urtica dioica fruit are a particularly significant indicator. It is a nitrophilous species and its presence suggests a heightened content of nitrogen in the soil. In the case of fen peatlands a higher concentration of this element is a result of peat mineralization (moorshing process), and the process occurrs during drying out the surface layers of peat deposits (GOTKIEWICZ 1987, 2007; TURBIAK and MIATKOWSKI 2006).

Conclusions

1. The moorsh layer of the studied profiles had seeds and fruit of 69 plant species, of which 42 species are peat-forming and 27 are non peat-forming species occurring on degraded and dried out peatlands.

2. The presence of *Urtica dioica* fruits and/or *Juncus effusus* and *J. conglomeratus* seeds is an important indicator of an ongoing decaying process.

3. The presence of *Triforium repens* seeds and *Alopecurus pratensis, Anthoxanthum odoratum* caryopses is an indicator of previous peatland usage as meadows.

4. The recorded non peat-forming species belong to 9 phytosociological classes, of which *Molinio-Arrhenatheretea* is the most numerous.

5. Each of the dried peatlands has its own composition of non peat-forming species, whose seeds or fruit are present in the moorsh layer.

6. The composition and number of non peat-forming species recorded in the moorsh layer of particular sites is not connected with the peat type, from which the moorsh originates and geographical location of peatland. The same lack of dependency occurs with regard to the number of seeds and fruit of particular plant species.

7. The presence of seeds or fruit of any of the recorded 27 non peat-forming plant species is always an indicator of the degradation in the upper layers of peat deposits.

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HISTOLOGICAL ANALYSIS OF ORGANOGENESIS AND SOMATIC EMBRYOGENESIS DURING SHOOT FORMATION IN SUGAR BEET (*BETA VULGARIS* L.) VIA GYNOGENESIS

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K e y w o r d s: sugar beet, organogenesis, somatic embryogenesis, gynogenesis, plant growth regulators.

List of abbreviations: $AgNO_3$ – silver nitrate, BAP – 6-benzylaminopurine, GA_3 – gibberellic acid, IBA – indole-3-butyric acid, KIN – kinetin, LS – Linsmaier and Skoog medium, MS – Murashige and Skoog medium, NAA – 1-naphthaleneacetic acid, PEG – polyethylene glycol, IAA – indoleacetic acid, PG_{0B} – De Greef and Jacob medium, TDZ – thidiazuron, TIBA – 2,3,5-triiodobenzoic acid, 2,4D – 2,4-dichlorophenoxyacetic acid.

A b s t r a c t

The aim of the present research were histological analysis of regenerating structures through *in vitro* gynogenesis from unfertilized ovules of sugar beet (*Beta vulgaris* L.). The process of shoot regeneration using a novel two stage method combines the preculture in liquid medium with the culture on solid medium. The highest number of explants that formed shoots (60%) was observed on medium supplemented with BAP, sucrose and gerlite, and as regards carbohydrates used in the medium most of explants forming shoots (42%) and the largest total number of shoots (110) was observed for glucose. To accurately determine the course of shoot formation, histological analyses were performed. Careful histologic evaluation of regenerating structures revealed the presence of numerous meristematic centres. In some meristems formation of specialized tissues and organs was observed, including epidermis, apical meristem, leaf primordia and tracheal elements. The analyses showed that the regeneration of the new structures from sugar beet ovules occurred both through organogenesis as well as somatic embryogenesis since the presence of somatic embryos in the globular stage or torpedo stage were observed.

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ANALIZA HISTOLOGICZNA PROCESÓW ORGANOGENEZY I SOMATYCZNEJ EMBRIOGENEZY PODCZAS FORMOWANIA PĘDÓW BURAKA CUKROWEGO (*BETA VULGARIS* L.) NA DRODZE GYNOGENEZY

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S ł o w a k l u c z o w e: burak cukrowy, organogeneza, somatyczna embriogeneza, gynogeneza, roślinne regulatory wzrostu i rozwoju.

A b s t r a k t

Celem przedstawionych badań była ocena histologiczna regenerujących struktur w trakcie procesu gynogenezy *in vitro* z niezapłodnionych zalążków buraka cukrowego (*Beta vulgaris* L.). Proces regeneracji pędów zachodził z wykorzystaniem nowatorskiej dwuetapowej metody łączącej prekulturę na pożywce płynnej z kolejną fazą prowadzoną na pożywce stałej. Najwyższą liczbę eksplantatów, które formowały pędy, obserwowano na pożywce uzupełnionej BAP, sacharozą i gerlitem, a spośród zastosowanych w pożywce węglowodanów najwięcej eksplantatów tworzących pędy i największą łączną liczbę pędów odnotowano dla glukozy. Przeprowadzono analizy histologiczne, aby precyzyjnie opisać drogę formowania się tych organów. W ocenie mikroskopowej regenerujących struktur wykazano obecność licznych centrów merystematycznych. W niektórych merystemach obserwowano sukcesywne fazy różnicowania, czego efektem było powstawanie wyspecjalizowanych tkanek i organów, m.in. epidermy, merystemu wierzchołkowego, primordiów liściowych, elementów trachealnych. W trakcie obserwacji stwierdzono, że regeneracja nowych struktur z zalążków buraka cukrowego zachodzi zarówno na drodze organogenezy, jak też somatycznej embriogenezy, odnotowano bowiem obecność zarodków somatycznych w stadium globularnym lub w stadium torpedy.

Introduction

Sugar beet (*Beta vulgaris* L.) is a species considered to be difficult to regenerate *in vitro*, however, its great economic importance determines an intensive research development to provide effective methods for propagation of plants from tissue and cell explants. The presented research analyses the morphogenetic potential of various tissues to be stimulated to form shoots, embryos or callus tissue under relevant factors. Direct organogenesis occurred on the apical parts of seedlings (MIEDEMA 1982, SULLIVAN et. al. 1993, RADY 1998), petioles (DETREZ et. al. 1989, KRENS and JAMAR 1989, GRIEVE et al. 1997), laminas (MIKAMI et. al. 1989, DOLEY and SAUNDERS 1989, OWENS and EBERTS 1992), apical meristems of storage roots and flower shoots (GOŚKA and ROGOZIŃSKA 1988, MAJEWSKA-SAWKA and JASSEM 1988, ZHONG et. al. 1993). The formation of callus tissues and indirect organogenesis were induced on e.g., the explants of cotyledons (KRENS and JAMAR 1989) and hypocotyls (JACQ et. al. 1992). In this research the primary explant was made up of unfertilized

ovules the shoots were produced from as a result of gynogenesis. To optimize the regeneration process, a two-stage culturing method was implemented; applying explant preculture in liquid medium, followed by tissue development and shoot differentiation on MS regeneration media with different amounts of solidifying substances, growth regulators and carbohydrates. The innovative sugar beet shoot regeneration method from unfertilised ovules facilitates an effective shoot formation irrespective of the season and the flowering stage. Histological analyses of regenerating structures make it possible to determine finally whether beet gynogenesis regeneration occurs thorough direct organogenesis or indirect organogenesis with callus or via somatic embryogenesis.

Materials and Methods

The explants applied to initiate the experiment were unfertilized ovules isolated from generative shoots of sugar beet plants (*Beta vulgaris* L.*)* grown *in vivo* 5 cm sections (Figure 1*a*) were sterilized for 1 min in 70% ethanol and for 20 min in 3% calcium hypochlorite and, in the final stage, they were rinsed in sterile distilled water. Ovules were isolated from 3–4 closed flower buds (Figure 1*b*) found on the shoot right above a bud at anthesis. The isolated ovules culture was maintained in liquid MS (MURASHIGE and SKOOG 1962) medium with 4.4 μmol dm^{-3} BAP. Thirty ovules were placed in each Erlenmeyer flask containing 25 ml of the medium. The material was shaken with the shaker (Lab Line Instruments, USA) at 150 rpm, 24°C and exposed to 16-hour photoperiod, at light intensity of about 40 μmol m^{-2} s^{-1}. After 12 weeks of culture in liquid MS medium supplemented with 4.4 μmol dm^{-3} BAP, the regenerating structures (3 replications, 20 explants each) – Figure 1*c*, were passaged onto solid MS media with a modified composition (Figure 1*d*, Table 1). In each variant 20 explants were inoculated on the growth medium, the experiment was repeated three times. Development of shoots formation (Figure 1*e*) and callus (Figure 1*f*) were observed.

Tissues developing on the regeneration media were collected and fixed to prepare microscope slides. Four structures were analysed in each of the 30 combinations, about 3 weeks after they were passaged from the liquid culture onto the solid media. The fixation occurred in 4% paraformaldehyde (PFA) and 0.5% glutaraldehyde in PIPES buffer, pH 7.3. The fixer was eluted from plant tissues in 0.05% PIPES buffer. In the next stage, tissues were dehydrated in ethanol solutions with increasing concentration from 30% to 100%, and supersaturated with a mixture of anhydrous ethanol and xylene in the ratio 3: 1, 1: 1, 1: 3, and placed in pure xylene. The final step involved a progressive supersaturation with Paraplast (Sigma Aldrich, USA) and polymerization.

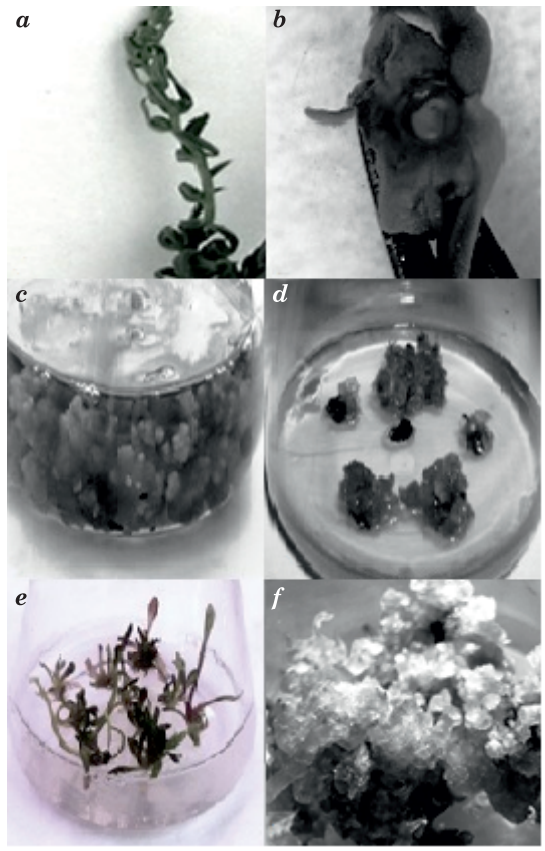

Fig. 1. Stages of sugar beet: *a* – cut-off generative soots fragments; *b* – a flower receptacle with isolated ovule; *c* – regenerating ovules over the preculture in liquid MS medium; *d* – regenerating ovules on solidified MS medium with 0.4% gerlite; *e* – regeneration shoots of sugar beet; *f* – callus tissue on solidified MS medium

The plant material immersed in Paraplast was cut with RM 2155 microtome (Leica Microsystems, Nussloch GmbH, Germany) into sections 10 μm thick, placed on slides coated with an aqueous polylysine solution (Sigma Aldrich, USA). To remove Paraplast from the surface and to hydrate the tissues, the slides were incubated in 100% xylene solutions and ethanol solutions at the concentration from 100% to 10% and in distilled water. After drying, the sections were stained with 0.5% methylene blue solution, 0.5% toluidine blue and 1.0% Azure II in 1.0% borax. The sections were coated with Vecta Mound resin (Vector Laboratories, USA), while histological analyses were performed using Jenalumar 2 light microscope (Carl Zeiss, Germany).

Table 1

Murashige and Skoog (MS) medium modifications applied in the experiment

Medium number	Concentration and type of medium components used				Other
	growth regulators		carbohydrates [mol dm^{-3}]	solidifying substance [%]	
	cytokinins [μmol dm^{-3}]	auxins [μmol dm^{-3}]			
1.	–	–	0.09 sucrose	0.7 agar	–
2.	4.4 BAP	–	0.09 sucrose	0.7 agar	–
3.	4.4 BAP	–	0.09 sucrose	0.4 agar	–
4.	4.4 BAP	–	0.09 sucrose	0.2 agar	–
5.	4.4 BAP	–	0.09 sucrose	0.4 gerlit	–
6.	4.4 BAP	–	0.06 sucrose	0.7 agar	–
7.	4.4 BAP	–	0.09 glucose	0.7 agar	–
8.	4.4 BAP	–	0.09 fructose	0.7 agar	–
9.	4.4 BAP	–	0.09 maltose	0.7 agar	–
10.	4.4 BAP	–	0.09 sucrose 0.09 mannitol	0.7 agar	–
11.	4.4 BAP	–	0.09 sucrose 0.09 fructose	0.7 agar	–
12.	0.44 BAP	–	0.09 sucrose	0.7 agar	–
13.	2.2 BAP	–	0.09 sucrose	0.7 agar	–
14.	0.44 BAP	0.44 NAA	0.09 sucrose	0.7 agar	–
15.	2.2 BAP	0.44 NAA	0.09 sucrose	0.7 agar	–
16.	4.4 BAP	0.44 NAA	0.09 sucrose	0.7 agar	–
17.	0.44 KIN	0.44 NAA	0.09 sucrose	0.7 agar	–
18.	2.2 KIN	0.44 NAA	0.09 sucrose	0.7 agar	–
19.	4.4 KIN	0.44 NAA	0.09 sucrose	0.7 agar	–
20.	0.44 KIN	0.44 2.4-D	0.09 sucrose	0.7 agar	–
21.	2.2 KIN	0.44 2.4-D	0.09 sucrose	0.7 agar	–
22.	4.4 KIN	0.44 2.4-D	0.09 sucrose	0.7 agar	–
23.	1.0 TDZ	1.0 IBA	0.09 sucrose	0.7 agar	–
24.	3.0 TDZ	1.0 IBA	0.09 sucrose	0.7 agar	–
25.	5.0 TDZ	1.0 IBA	0.09 sucrose	0.7 agar	–
26.	4.4 BAP	–	0.09 sucrose	0.7 agar	1.0 μmol dm^{-3} TIBA
27.	4.4 BAP	–	0.09 sucrose	0.7 agar	2.0 μmol dm^{-3} TIBA
28.	4.4 BAP	–	0.09 sucrose	0.7 agar	0.025 mol $dm^{-3}AgNO_3$
29.	4.4 BAP	–	0.09 sucrose	0.7 agar	40 g dm^{-3} PEG
30.	4.4 BAP	–	0.09 sucrose	0.7 agar	0.5% activated carbon

Results and Discussion

The results of numerous experiments with sugar beet regeneration *in vitro* confirm that shoot formation via organogenesis or somatic embryogenesis is hardly efficient and the final effects have been considered as unsatisfactory (TETU et. al. 1987, DETREZ et. al. 1988, 1989, TENNING et. al. 1992). The study analysed the morphogenetic potential of different tissues which, when exposed to relevant factors, can be stimulated to form shoots, embryos or callus tissue. It has been shown that regeneration efficiency, evaluated from the number of shoots derived from a specific pool of initial tissue, depends on the explant

type and origin. To determine the course of shoot formation accurately, histological analyses of ovules sampled in the adequate development stage were made. Buds above the bud at anthesis stage, i.e. when an embryonic sac was fully formed, were isolated. Available literature indicates a dependence of gynogenetic process efficiency on the donor plant condition, flowering phase and on the season. As provided by DOCTRINAL et. al. (1989), June is a favourable time for ovules isolation. Effective ovule regeneration is also possible by isolating explants from May to September (LUX et. al. 1990). The reaction of ovules to *in vitro* conditions also depends on plant growth phase; it has been documented that explants isolated at the end of flowering show a reduced ability to differentiate (GOŚKA 1997). The morphogenetic potential of tissues from ovule cultures was estimated after four weeks of development on regeneration media. The numbers of developing explants and the explants forming shoots were specified (Table 2). The most intense regeneration was observed on the medium supplemented with 4.4 μmol dm^{-3} BAP, 0.09 mol dm^{-3} sucrose and 0.4% gelrite. On that medium, explants formed shoots (60%) showing numerous leaves with properly formed lamina vivid green in colour. Differentiated capacity to gynogenesis in sugar beet, depending on the applied cytokinins, and their concentration, was also shown by PAZUKI et. al. (2017). As part of the experiments, the effects of monosaccharides (glucose, fructose), disaccharides (sucrose, maltose), and polyhydric sugar alcohol, mannitol, on morphogenesis during the first four weeks of culture were compared. The best effect was observed for 0.09 mol dm^{-3} glucose as 25 explants (42%) formed shoots with the adequate plant habit on that medium whereas, when exposed to maltose, the number was 8 (13%) only. A low differentiation efficiency was also noted on the medium with sucrose and fructose, on which only 9 explants (15%) formed shoots with a habit typical for sugar beet. Shoot formation efficiency was analysed after the third passage onto regeneration media; after 12 weeks of explant (shoots) development on 30 solid media of different composition (Table 1). When 0.09 mol dm^{-3} sucrose, commonly applied in the medium, was replaced with glucose, the number of formed shoots has doubled (110). The increased intensity of organogenesis was also observed when sucrose concentration was reduced from 0.09 mol dm^{-3} to 0.06 mol dm^{-3}. When exposed to maltose and sucrose with mannitol, differentiation processes were less intensive. An analysis of the effect of sugars on regeneration was also carried out by TEIXEIRA da SILVA (2004). Chrysanthemum plants most efficiently regenerated in the presence of sucrose, glucose and fructose, while a much lower effect on processes in cultures *in vitro* was identified for the other sugars analysed (e.g. maltose, mannose, galactose, mannitol). Differences in carbohydrates effectiveness for the processes of differentiation and regeneration of cells in cultures *in vitro* are due to the fact that the plants are not able to metabolize all the carbohydrates contained in the medium with the same intensity.

Table 2
Efficiency of shoots and callus formation depending on the regeneration medium composition

Medium number*	Number of regenerated explants	The number of explants forming shoots	The number of explants forming callus
1	19	17	2
2	25	19	6
3	30	25	5
4	20	12	8
5	36	36	0
6	23	20	3
7	28	25	3
8	13	10	3
9	9	8	1
10	10	10	0
11	10	9	1
12	18	16	2
13	7	6	1
14	8	8	0
15	14	12	2
16	9	9	0
17	20	9	11
18	23	19	4
19	18	14	4
20	45	8	37
21	54	17	37
22	50	10	40
23	10	6	4
24	6	6	0
25	12	7	5
26	18	18	0
27	10	10	0
28	36	20	16
29	10	0	10
30	0	8	2

Explanations: *numbers 1 to 30 correspond to the numbers of media as given in Table 1.

From about 120 tissue sections, 960 histological sections were produced which, after removing paraffin wax and staining, were observed with the light microscope. It was found that organogenesis from ovule explants with the two-phase method was indirect, as relatively large undifferentiated callus cells (Figure 2*a*, 2*b*). Sugar beet plant organogenesis via callus was also noted on the explants of cotyledons (KRENS and JAMAR 1989) and hypocotyl (JACQ et. al. 1992). As reported in literature, direct organogenesis occurred on the apical parts of seedlings (MIEDEMA 1982, SULLIVAN et. al. 1993, RADY 1998), petioles (DETREZ et. al. 1989, KRENS and JAMAR 1989, GRIEVE et. al. 1997), laminas (MIKAMI et. al. 1989, DOLEY and SAUNDERS 1989, OWENS and EBERTS 1992),

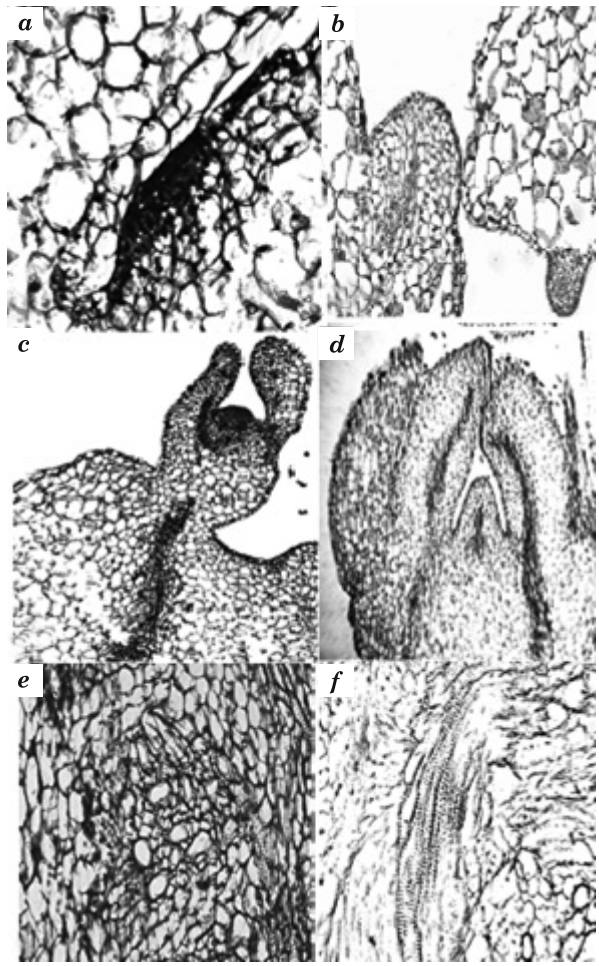

Fig. 2. Differentiating tissues of sugar beet: *a* – meristematic cells located between the callus cells (200x); *b* – differentating callus tissue containing primordial meristems, visible layer of epidermal cells (100x); *c* – apical meristems in *in vitro* ovules culture with primordial leaves (100x), *d* – apical meristems in *in vitro* ovules culture with primordial leaves (200x); *e* – elements of vascular tissue in callus, cross-section (200x); *f* – longitudinal section (200x).

apical meristems of storage roots and flower shoots (GOŚKA and ROGOZIŃSKA 1988, MAJEWSKA-SAWKA and JASSEM 1988, ZHONG et. al. 1993).

A thorough histological evaluation of regenerating sugar beet structures revealed the presence of numerous meristematic centres containing small, closely adjacent and intensely coloured cells. It was found that adventitious meristems were formed both on the surface of callus, as well as in its deeper parts. In some meristems there were successive phases of differentiation,

which resulted in a formation of specialized tissues and organs, including epidermis (Figure 2*b*), apical meristem, leaf primordia (Figure 2*c*, 2*d*) and tracheal elements (Figure 2*e*, 2*f*). The processes of differentiation were intensified by the presence of BAP (4.4 μmol dm^{-3}) and 2,4-D in medium, which, in turn, increased the number of forming specialized tissue structures. Differentiated capacity to regenerate ovules was also shown by GOŚKA (1997). Of all the genotypes studied, the best results were recorded on the medium containing BAP in combination with NAA. Other authors found that in the absence of cytokinins, differentiation and regeneration processes can be inhibited (DOCTRINAL et. al. 1989). In this experiment, the effect of synthetic auxin 2,4-D on differentiation processes was also tested. Its presence enhanced the formation of callus, which is consistent with the observations of VAN GEYT et. al. (1987) and D'HALLUIN and KEIMER (1986). Intensification of callus formation in the presence of 2,4-D is due to a strong influence of the regulator on dedifferentiation of cells and their acquisition of meristematic features. A possible stimulation of meristematic cell division in explant is also suggested (ORLIKOWSKA 1997).

The histological observations of regenerating structures revealed the presence of somatic embryos in the globular stage (Figure 3a) or torpedo stage (Figure 3*b*). The structures in the torpedo stage showed a clear bipolarity, with visible shoot and root regions (Figure 3*b*). A thorough analysis of serial sections suggests that embryos were formed from the epidermal and subepidermal layers as they were connected to the tissue with only a few cells, or a much larger group of cells (Figure 3*c*). Somatic embryos were formed only on 3 out of 30 regeneration media used: 2.2 μmol dm^{-3} BAP and 0.44 μmol dm^{-3} NAA, 0.44 μmol dm^{-3} KIN and 0.44 μmol dm^{-3} NAA as well as 0.44 μmol dm^{-3} KIN and 0.44 μmol dm^{-3} 2,4-D. Each medium contained 0.09 mol dm^{-3} sucrose and 0.7% agar. The histological analyses described above showed that regenerating new structures from sugar beet ovules occurred both via organogenesis as well as via somatic embryogenesis, as evidenced by GOŚKA and JASSEM (1988), however, embryos formation was observed only on the media supplemented with cytokinin and auxin. TETU et. al. (1987) also reported on embryogenesis processes initiation on petiole sections placed on media, supplemented with phytohormones of both groups. The presence of BAP and IBA was also essential for somatic embryogenesis induction in the explants of six sugar beet lines tested by FREYTAG et. al. (1988) and TETU et. al. (1987). The formation of somatic embryos during explant *in vitro* culture sometimes requires successive passages onto media with different amounts of micro- and macroelements as well as hormones. Media used at the beginning of culture usually contain high rates of growth regulators, in contrast to the media where the embryo differentiation and development occurs. PEDERSEN and ANDERSEN

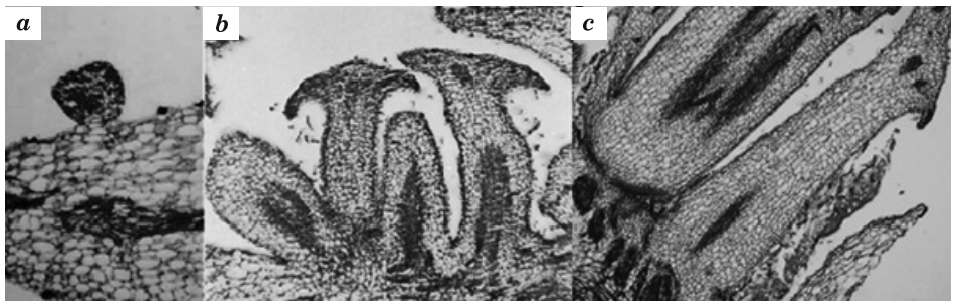

Fig. 3. Somatic embryos of sugar beet in various stages of development: *a* – somatic embryos of sugar beet in globular stage of development (100 x); *b* – somatic embryos of sugar beet in torpedo stage of development with visible shootsand root zone (100 x); *c* – the connection method of somatic embryos from stem tissue (100x).

(1988) induced the processes of sugar beet embryogenesis exposed to high concentrations of cytokinin BAP on PGoB medium (DE GREEF and JACOBS 1979) and succeeded in embryo multiplication on MS medium supplemented with low rates of BAP, IAA and GA3. Embryogenic callus was also produced from the apical parts of generative shoots (KUBALKOVA 1990) after placing the tissues sections on media MS, PGoB or LS (LINSMAIER and SKOOG 1965). The author analysed the impact of media supplemented only with cytokinins or not containing growth regulators on morphogenetic callus properties. Embryogenic ability persisted as a result of culture on the media containing 2.5 μmol dm^{-3} BAP and 2.5 μmol dm^{-3} KIN and, over three years, on phytohormone-free medium. Somatic sugar beet embryos can form from callus cells on leaf disc explants placed on phytohormone-free media (DOLEY and SAUNDERS 1989). The same type of explant was used for inducing somatic embryos of beet lines *Rel-1* (TSAI and SAUNDERS 1995). Some authors induced embryogenesis in two stages. The first involved an initial callus tissue incubation in liquid media, in the dark, and the second one – culture exposed to light, on solid media. The most effective regulator combination was a combination of 1 mg dm^{-3} NAA and 0.1 mg dm^{-3} ABA (TSAI and SAUNDERS 1995). Positive effects of 2,3,5-triiodobenzoic acid (TIBA) on the initiation and differentiation of somatic embryos from callus are also suggested (TETU et. al. 1987, DOLEY 1990). Callus tissue forming on leaf sections derived from seedlings grown in the presence of TIBA demonstrated a greater potential for development of somatic embryos than callus of control plants (MOGHADDAM and MESBAH 2000). Somatic beet embryogenesis can be induced not only from suspension or callus cells but also from mature or not fully mature zygotic embryos. Such explants were previously applied as a stock material for somatic embryogenesis induction in cereals (VASIL 1988), conifers (TAUTORUS et. al. 1991), arabidopsis

(GAJ 2002) and rose (KIM et. al. 2003). According to the studies available, growth regulators are essential for somatic embryogenesis induction in cultured zygotic embryos of sugar beet, and an especially intensive effect is reported for synthetic auxin 2,4-D (AMMIRATO 1983, TENNING et. al. 1992). The formation of somatic embryos was also induced in the cultured cotyledons isolated from mature sugar beet embryos. Incubation of primary explants occurred on PGOB medium containing BAP and 2,4-D, and the embryogenic properties were induced on MS medium in the presence of BAP and TIBA (KULSHRESHTHA and COUTTS 1997). A genotype is an important factor for the course of somatic embryogenesis. The experiment results reported earlier show that the formation of embryos via somatic embryogenesis was successful only for a few genotypes (KUBALAKOVA 1990, TENNING et. al. 1992, TSAI and SAUNDERS 1995, KULSHRESHTHA and COUTTS 1997, GOŚKA 2001, 2002). The ability of explants to initiate embryogenesis also depends on the embryo development stage. TENNING et. al. (1992) report on embryos collected from 10 to 20 days after anthesis, and the highest somatic embryos formation potential was found for embryos with underdeveloped cotyledons, classified as immature embryos. Those histological analyses for sugar beet tissues formed via gynogenesis showed that regeneration of new structures from sugar beet ovules occurs both via organogenesis and via somatic embryogenesis. This method of producing beet plants as a result of ovule culture *in vitro* has both advantages and disadvantages, as compared with the earlier techniques using the same explants. A possibility of producing and, in fact, of unlimited multiplication of initial tissues in liquid cultures, which can be induced to form shoots at any time of the year, is definitely an advantage. A genetic diversity of the regenerates and a difficulty in identifying gynogenetic embryos as well as determining their development path is a major drawback. To improve this method, further research should aim at eliminating the unwanted genetic variation. One way to solve this problem could be to eliminate the participation of callus and to induce direct organogenesis. In the light of the observations it seems to be relevant to examine other genotypes to confirm the method effectiveness and factors acting on explant in *in vitro* cultures: culture duration, the number of passages, medium type and its solidification level as well as the type and concentration of the phytohormones used.

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ANALYSIS OF THE SIZE OF FAT GLOBULES IN MILK AND CREAM DISPERSED IN DIFFERENT REAGENTS SOLUTIONS

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K e y w o r d s: milk, cream, fat globule, size distribution, laser diffraction.

A b s t r a c t

This study compared the dimensions of the dispersion of fat globules in dairy products based on microscopic and instrumental methods using laser diffraction. The research involved an analysis of 20% and 10% fat cream and 3.5% fat milk, both non-homogenized and homogenized at 20 MPa and 100 MPa. Chemical compounds affecting the dissociation or disaggregation of casein micelles and fatty globules agglomerates were added to samples intended for instrumental measurements. It was found that those compounds did not have any significant effect on changes of determinants characterizing the dispersion of fat globules in cream, while in milk they determined the size of particles with a decreasing intensity of differences between the parameters under analysis in the following order: non-homogenized milk > milk homogenized at 20 MPa > milk homogenized at 100 MPa.

ANALIZA ROZMIARÓW KULECZEK TŁUSZCZOWYCH W MLEKU I ŚMIETANCE ZMIESZANYCH Z ROZTWORAMI RÓŻNYCH ZWIĄZKÓW CHEMICZNYCH

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S ł o w a k l u c z o w e: mleko, śmietanka, kuleczka tłuszczowa, rozkład wielkości, dyfrakcja laserowa.

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Abstrakt

Praca dotyczy porównania wielkości charakteryzujących dyspersję kuleczek tłuszczowych w produktach mleczarskich określonych w oparciu o wyniki metody mikroskopowej oraz instrumentalnej z wykorzystaniem dyfrakcji laserowej. Badano śmietankę o zawartości tłuszczu 20% i 10% oraz mleko o zawartości tłuszczu 3,5% niepoddane i poddane homogenizacji w ciśnieniu 20 i 100 MPa. Do próbek przeznaczonych do pomiaru instrumentalnego zastosowano dodatek związków chemicznych wpływających na dysocjację lub dysagregację micel kazeinowych oraz aglomeratów kuleczek tłuszczowych. Stwierdzono, że związki te nie wpłynęły znacząco na zmiany wyróżników charakteryzujących dyspersję kuleczek tłuszczowych w śmietance. W mleku natomiast determinowały rozmiar cząstek z malejącym nasileniem różnic analizowanych parametrów w następującej kolejności: mleko niehomogenizowane > mleko homogenizowane w ciśnieniu 20 MPa > mleko homogenizowane w ciśnieniu 100 MPa.

Introduction

Milk and cream are examples of naturally occurring oil-in-water emulsions, in which the dispersed phase is fat in the form of membrane-covered globules and the continuous phase is milk plasma with its components. The size distribution of fat globules, native or subject to technological processes, is one of their main properties. A depedence exists between the degree of fat dispersion and its content in raw milk, according to which the size of fat globules increases along with an increase in the fat content (KIEŁCZEWSKA et al. 2008, WIKING et al. 2004). It should be noted that an increase in the dimensions of fat globules can contribute to a change in the fatty acid content, which may have technological consequences (MICHALSKI 2004, MICHALSKI et al. 2004).

The size of fat globules in milk and cream is affected by the processes applied during milk processing (mainly homogenization) which results in reduction and standardization of the size of fat globules and their even dispersion over the entire volume of the sample. Properly homogenized milk is characterized in normal conditions by an equal fat content over the entire sample, which determines the stability of the emulsion, mainly protecting it against creaming, but also flocculation, coalescence and demulsification of fat in the final product (KIEŁCZEWSKA and KRUK 1997, KOWALIK 2011). Determination of fat globule size is one of the determinants helping to work out optimal parameters for homogenization of a given product, as well as to carry out an evaluation of its efficiency directly after production and during storage. For the above reasons, it is important from the application point of view to control the sizes of the dispersed phase in milk and cream.

A traditional technique used to determine the sizes of fat globules is the microscopic method, based on their observation and measurement of their diameters. A microscopic observation provides comprehensive information concerning the dispersion of milk fat, as well as the occurrence and identifica-

tion of certain types of emulsion instability, e.g. flocculation or disruption of fat globule membranes. Unquestionable scientific and technical progress in the field of evaluating the size of fat globules in milk and cream, also used for assessment of dispersion of other milk components, has extended cognitive possibilities with new measurement techniques. The methods that are most frequently applied during the analysis of fat globules and other milk particles are laser diffraction techniques, determining the size of particles by measuring intensity of scattered light. A particle-size analyzer based on laser diffraction does not measure the size of particles, but measures the intensity of laser light scattered at small angles for large participles and at large angles for small particles, which is then converted into the size of particles over the broad range of values. Therefore, interpretation of the light intensity spectrum requires conversion of light dispersion data into particle size, which is conducted on the basis of the Mie theory and pursuant to its assumptions.

The analysis of particle size with the use of the instrumental method involves all particles over a given range, also including their agglomerates (apart from single fat globules) which are treated as one particle, as well as casein micelles. The accuracy and reproducibility of the obtained results and their proper interpretation requires a specific methodology of preparing samples for measurements. The method of preparing milk emulsions for fat globule measurement can reduce the possibility of false analysis based on measuring the size of particles with indirect methods. Literature data provide various methods used for preparing milk samples for measurement purposes (BERTON et al. 2011, CANO-RUIZ and RICHTER 1997, HAYES and KELLY 2003, HUPPERTZ et al. 2003, MCCRAE and LEPOETRE 1996, MICHALSKI et al. 2001a, 2001b, RONHOLT et al. 2012, THIEBAUD et al. 2003). Generally, these involve the application of chemical compounds with double effect on dissociation or disaggregation of casein micelles: 1) it eliminates them during measurement of particles and 2) helps to disintegrate fat globule agglomerates, which enables measurement of individual fat globules. Considering the above aspects, this study undertakes an attempt to select an efficient methodology and to specify the procedure for preparing milk and cream samples for measurements of fat globule size, which would eliminate any irregularities occurring in the measurements carried out with the instrumental method.

The aim of the study was to compare determinants of fat globule dispersion based on microscopic observations and instrumental measurements, depending on the methodology used for preparing milk and cream samples.

Material and Methods

Research material

The research material was bovine milk originating from the Bałdy Teaching and Research Station of the University of Warmia and Mazury in Olsztyn. Raw milk intended for research satisfied the requirements of the Regulation of the Ministry of Agriculture and Rural Development (Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi... Dz.U. z 2004 r. nr 188, poz. 1946) in terms of its cytological quality (somatic cell count $\leq 4 \cdot 10^5$ cm^{-3}) with Fossomatic 5000 apparatus (Foss, Hillerod, Dania), microbiological quality (total count of microorganisms $\leq 10^5$ cfu cm^{-3}) with Bactoscan 8000S apparatus (Foss, Hillerod, Dania), freezing point ($\leq$ -0.512°C) with Krioskop 800 cL apparatus (TridentMed, Warsaw, Poland), density ($\geq$ 1.028 g cm^{-3}) with a lactodensimeter, non-fat dry matter content ($\geq$ 8.5%) and protein content ($\geq$ 3.2%) using a MilcoScan 4000 apparatus (Foss, Hillerod, Denmark).

Organization of the experiment

The research involved products of varied fat content and of different size of fat globules. Milk was centrifuged at 45°C in a skimming centrifuge (Gea Westfalia Separator System GmbH, Oelde, Germany) and the obtained skimmed milk and cream were used for fat content standardization to 20% and 10% in cream and 3.5% in milk. Two-thirds of the 3.5% fat milk volume was heated to 60°C and homogenization was conducted at 20 MPa and 100 MPa with a PandaPlus 2000 homogenizer (Gea Niro Soavi, Parma, Italy). Milk and cream intended for analyses were preserved by an addition of 2% NaN_3 solution in the amount of 1 cm^3 1 dm^{-3}. During the study, milk and cream were stored in a refrigerator at about 4°C. Before the determinations, samples were heated up to 20°C and carefully stirred, after which they were left for about 1 hour for component arrangement and the physico-chemical properties to stabilize.

The experiment was conducted in triplicate.

Evaluation of determinants of fat globule dispersion by the microscopic method

The degree of milk fat dispersion in milk and cream was evaluated by the microscopic method using a set consisting of an optical microscope (Carl Zeiss, Jena, Germany) (63x magnification), CCD Moticam Pro camera (Motic, Motic

Asia, Hong Kong, China) and a monitor, by comparing fat globule diameters with the standard scale. Microscopic preparations of milk and cream samples were prepared according to the requirements specified in the *Mleko, śmietanka...* PN-A-86059:1975 standard, using an alcohol solution of 2-naphthalenol,1-[2-[4-(2-phenyldiazenyl)phenyl]diazenyl]. On the basis of results of the microscopic observations conducted (3 preparations, 5 fields of view in each of them), the percentage share of fat globules was calculated for specific size ranges, the mean diameter over surface area (Sauter Mean Diameter – SMD) $d_{32} = \Sigma d_i^3 n_i / \Sigma d_i^2 n_i$, the mean diameter over volume (De Brouckere Mean Diameter) $d_{43} = \Sigma d_i^4 n_i / \Sigma d_i^3 n_i$, where n_i number of fat globules of d_i diameter (THIEBAUD et al. 2003) and fat globule surface area of 1 cm^3 milk fat (specific surface area) ssa = $6/d_{32}$ (OORTWIJN and WALSTRA 1979]).

Evaluation of the determinants of fat globule dispersion by the indirect optical method using laser diffraction analysis

The analysis of the fat globule size with laser diffraction (indirect method) was carried out using a Mastersizer 3000 particle size analyzer equipped with an He-Ne laser (632.8 nm), with a Hydro EV dispersion unit (Malvern Instrument, Malvern, United Kingdom). The measurement was conducted for refractive indices of milk and water de-ionized with Milli-Q (Millipore, Molsheim, France) /SDS solutions as dispersant of 1.46 and 1.33, respectively, and obscuration ranging from 10% to 12%. On the basis of the obtained results, the following values were determined: d_v10 (diameter below which 10% particles of the entire distribution occur), d_v50 (diameter below and above which 50% particles occur), defined as the median of volume weighted particle size distribution, d_v90 (diameter below which 90% particles occur), d_{43}, d_{32}, ssa. Samples were prepared according to specific methodologies, according to which they were labelled with the following numbers:

1 – milk and cream without any additions (CANO-RUIZ and RICHTER 1997, HAYES and KELLY 2003);

2 – 3 volumes of milk or cream were mixed with 1 volume of 0.4 M trisodium citrate containing 4% SDS (HUPPERTZ et al. 2003);

3 – 0.2 volume of milk or cream in the proportion to 1 volume of 35 mM EDTA/NaOH, pH 7 per 100 ml water (BERTON et al. 2012);

4 – milk or cream was mixed in the 1:10 proportion with the solution containing 5 g SDS/L (THIEBAUD et al. 2003);

5 – 1 volume of milk or cream was mixed with 1 volume of 35 mM EDTA/NaOH, pH 7 containing 5 g l^{-1} Tween 20 (MCCRAE and LEPOETRE 1996).

The following reagents were used: ethylenediaminetetraacetic acid disodium salt (EDTA) (Chempur, Piekary Śląskie, Poland), sodium hydroxide (NaOH) (Chempur, Piekary Śląskie, Poland), 2-naphthalenol,1-[2-[4-(2--phenyldiazenyl)phenyl]diazenyl] (Merck, Darmstadt, Germany), trisodium citrate (Chempur, Piekary Śląskie, Poland), sodium azide (NaN_3) (Avantor Performance Materiale, Gliwice, Poland), Tween 20 (Sigma – Aldrich, Saint Louis, Missouri, USA), sodium dodecyl sulfate (SDS) (Lach-ner, Neratovice, Czech Republic).

The measurement was carried out after previous dilution in a diffractometer cell and the size of particles was measured using Standard Measurement Procedures of the Standard Operating Procedure established before performing analyses, to ensure circularity of measurement conditions. The measurements were conducted three times, with 5 measurements in each cycle.

Statistical analysis of results

The statistical analysis included determination of mean values and standard deviations and conducting the least significant differences (LSD) test at two levels of significance ($\alpha = 0.05$; $\alpha = 0.01$), using StatSoft Inc. Statistica v. 10.0 software (Tulsa, Oklahoma, USA).

Results and Discussion

The obtained results of microscopic observations and instrumental measurements demonstrated that an increase in fat content in the product was accompanied by an increase in the number of large globules and a decrease in the share of small globules, which was reflected in an increase in the average volume-surface diameter (d_{32}) and Brouckere diameter (d_{43}) and a reduction of the value describing the surface area of 1 cm^3 of milk fat (ssa). On the other hand, the application of pressure homogenization resulted in an increase in the share of small fat globules in 3.5% fat milk, which brought about a reduction in the value of parameters describing their size and an increase in their surface area in comparison to non-homogenized milk (Table 1, Figure 1, Figure 2).

Values describing the dispersion of fat globules in milk and cream obtained through the instrumental analysis significantly differed from the data obtained as a result of calculations based on the results of microscopic observations (Table 1). What should be emphasized is the fact that with the application

Table 1

Results of evaluation of emulsion phase dispersion measures for milk and cream

Sample		Measure	Microscopic method	Instrumental method				
				1	*2*	*3*	*4*	*5*
Cream with 20% fat content		D_{32} [μm]	8.21 ± 0.87^{aA}	2.45 ± 0.12^{bB}	2.55 ± 0.10^{bB}	2.42 ± 0.17^{bB}	2.42 ± 0.18^{bB}	2.30 ± 0.12^{bB}
		D_{43} [μm]	9.95 ± 0.92^{aA}	4.34 ± 0.60^{bcB}	4.25 ± 0.39^{bcB}	4.28 ± 0.31^{bcB}	4.42 ± 0.10^{bcB}	3.97 ± 0.12^{cB}
		Ssa [$m^2\,cm^{-3}$]	0.74 ± 0.08^{aA}	2.46 ± 0.12^{bcBC}	2.36 ± 0.09^{bcBC}	2.49 ± 0.18^{cBC}	2.49 ± 0.19^{bcBC}	2.61 ± 0.13^{cC}
Cream with 10% fat content		D_{32} [μm]	5.90 ± 0.49^{aA}	2.36 ± 0.06^{bB}	2.51 ± 0.05^{bB}	2.45 ± 0.14^{bB}	2.40 ± 0.17^{bB}	2.36 ± 0.10^{bB}
		D_{43} [μm]	7.47 ± 0.83^{aA}	3.88 ± 0.40^{bB}	4.05 ± 0.28^{bB}	4.10 ± 0.51^{bB}	3.90 ± 0.30^{bB}	3.87 ± 0.54^{bB}
		Ssa [$m^2\,cm^{-3}$]	1.02 ± 0.08^{aA}	2.55 ± 0.06^{bB}	2.39 ± 0.05^{bB}	2.46 ± 0.14^{bB}	2.51 ± 0.19^{bB}	2.55 ± 0.10^{bB}
Milk with 3.5% fat content	non--homogenized	D_{32} [μm]	5.44 ± 0.44^{aA}	1.51 ± 0.12^{cC}	2.30 ± 0.20^{bB}	2.25 ± 016^{bB}	1.66 ± 0.16^{cC}	2.25 ± 0.12^{bB}
		D_{43} [μm]	6.15 ± 0.63^{aA}	3.28 ± 0.25^{cB}	3.87 ± 0.32^{bcB}	3.82 ± 0.37^{bcB}	3.31 ± 0.22^{cB}	3.82 ± 0.43^{bcB}
		Ssa [m^2/cm^3]	1.21 ± 0.11^{aA}	3.98 ± 0.30^{bB}	2.63 ± 0.22^{cC}	2.68 ± 0.18^{cC}	3.64 ± 0.37^{bB}	2.67 ± 0.14^{cC}
	homogenized at 20 MPa	D_{32} [μm]	2.28 ± 0.24^{aA}	0.91 ± 0.03^{cB}	1.21 ± 0.21^{bB}	1.18 ± 0.16^{bcB}	1.07 ± 0.06^{bcB}	1.16 ± 0.13^{bcB}
		D_{43} [μm]	2.77 ± 0.25^{aA}	2.26 ± 0.12^{bBC}	2.72 ± 0.10^{aAB}	2.75 ± 0.24^{aA}	2.13 ± 0.19^{bC}	2.26 ± 0.22^{bBC}
		Ssa [m^2/cm^3]	2.65 ± 0.27^{aA}	6.62 ± 0.22^{cC}	5.05 ± 0.81^{bB}	5.16 ± 0.65^{bB}	5.62 ± 0.29^{bBC}	5.23 ± 0.53^{bB}
	homogenized at 100 MPa	D_{32} [μm]	1.65 ± 0.08^{aA}	0.63 ± 0.06^{bB}	0.67 ± 0.05^{bB}	0.68 ± 0.06^{bB}	0.66 ± 0.08^{bB}	0.65 ± 0.07^{bB}
		D_{43} [μm]	2.08 ± 0.08^{bB}	1.22 ± 0.07^{aA}	1.09 ± 0.07^{acAC}	1.06 ± 0.12^{cC}	1.03 ± 0.03^{cC}	1.03 ± 0.07^{cC}
		Ssa [$m^2\,cm^{-3}$]	3.63 ± 0.16^{bB}	9.52 ± 0.82^{aA}	8.94 ± 0.65^{aA}	8.88 ± 0.85^{aA}	9.12 ± 1.03^{aA}	9.29 ± 0.96^{aA}

$n = 3$; values in one row denoted with the same small letter are not significantly different at $\alpha = 0.05$, values in one row denoted with the same capital letter are not significantly different at $\alpha = 0.01$.

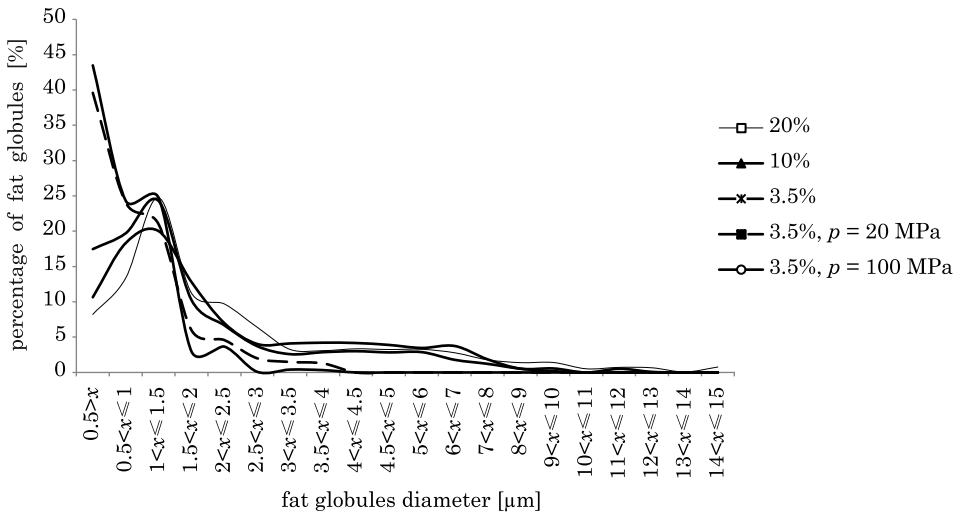

Fig. 1. Fat globules distribution in milk and cream (microscopic method)

of the microscopic method, the possibility of determining the fat globule size distribution in the analysed sample depends on magnification. At the magnification used for microscopic examinations, no fat globules of small dimensions were observed. Mastersizer 3000, a fat globule size analyser is equipped with a red and blue laser to measure both large and small particles. In tests applying laser diffraction, the smallest fat globules are also counted, as well as particles of a size typical for casein micelles. On the other hand, a microscopic observation of fat globules is an analysis providing comprehensive information concerning not only the dispersion of fat globules, but also the occurrence and identification of emulsion destabilisation forms. The analysis of microscopic images permits to predict the layer of fat and to determine the occurrence of fat globule flocculation and disruption of fat globule membranes with the formation of free fat. Although the results obtained with the two methods differed, they were characterized by a similar trend of changes regarding the size of fat globules depending on the fat content in the sample and the applied pressure of the homogenization process. These results were confirmed by the results of studies conducted by OLSON et al. (2004), who found that the size of fat globules decreased with a decrease in the fat content in milk and cream. They further recorded that emulsion phase dispersion increased in a 3.5% fat milk as a result of microfluidisation, which was most significant within the pressure range of 50–100 MPa, with an increase in particle size above this range.

The results of measurements obtained by the instrumental method demonstrated that the application of chemical reagents at the stage of preparing

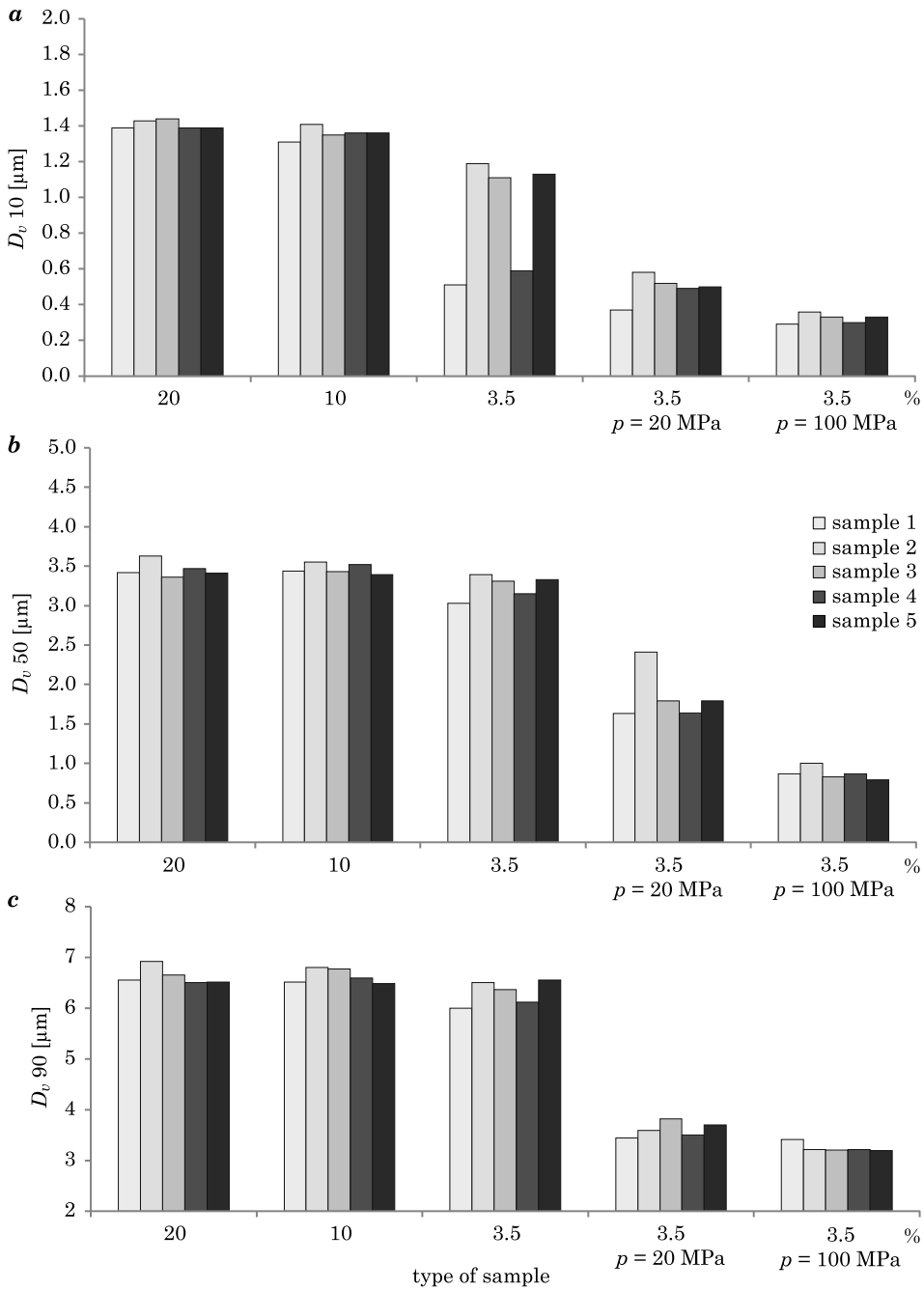

Fig. 2. Comparison of diameters of fat globules in milk and cream determined by the instrumental method, depending on sample preparation

samples for analyses affected the changes in particle size determinants in the analysed samples in the direction and within the range depending on their fat content and the homogenization process, although in most cases it contributed to an increase in particle size in comparison to corresponding samples measured without any previous preparation (Table 1, Figure 2). The obtained higher values of d_{43} in comparison to d_{32} of fat globules (Table 1) could indicate the presence of small amounts of large particles, which was confirmed in the d_v90 values for the dispersed phase of milk and cream (Figure 2*c*).

The diversity of results obtained for the analysed parameters describing the size of particles in milk and cream was determined by a dual effect of reagents applied at the stage of preparing the samples for particle measurements. The application of trisodium citrate and EDTA results in dissociation of casein micelles (WALSTRA 1990, HUPPERTZ et al. 2003, MCCRAE and LEPOETRE 1996) and, in consequence, they are not counted during the measurement of particle sizes. On the other hand, the applied non-ionic surfactant Tween 20 and anionic protein-denaturing SDS remove absorbed materials from the oil-water interphase surface and disturb the agglomeration of fat globules (DE FEIJTER et al. 1987), disrupt previously-formed fat globule agglomerates (THIEBAUD et al. 2003) and maintain the globules in a dispersed form, enabling size measurement of individual globules.

The method used for preparing samples before measurement carried out by the instrumental method was of low significance during the analysis of products with high fat content. This is proven by statistical differences that were insignificant at both significance levels occurring in most cases in d_{32} and d_{43} values for fat globules in cream with 10% and 20% fat content (non--homogenized). Nevertheless, the obtained reduction in values of the analysed determinants describing dispersion of the emulsion phase in 20% fat cream as a result of adding EDTA combined with Tween 20 at the stage of preparing samples for measurements, indicated its effect on fat globule agglomerate disintegration. For cream in which the share of fat was higher than the share of protein, the dimension of fat globules to a larger extent determined the size of measured particles. This was confirmed by a small increase in the d_v10 dimension of particles of 20% and 10% fat cream as a result of adding reagents dissociating casein micelles, amounting to 13% and 35%, respectively (Figure 2*a*). For a homogenized cream, the emulsion phase dispersion measures may probably be modified depending on the method of sample preparation prior to the measurement, which may be a subject of further research.

For non-homogenized milk with lower fat content and higher protein content (mainly casein) in comparison to cream, sample preparation before measurement was of crucial importance. As a result of applying reagents, changes in d_{32} and ssa dimensions were statistically significant at both levels

of significance, while the values of d_{43} did not statistically significantly differ at $\alpha = 0.01$, regardless of the method applied for preparing non-homogenized milk before instrumental measurements. No statistically significant effect at both levels of significance was found for application of SDS alone as regards the analysed values in non-homogenized milk.

For homogenized milk, modification of the colloidal phase as a result of homogenization, such as casein adsorption on the surface of homogenized fat globules (CANO-RUIZ and RICHTER 1997, KIEŁCZEWSKA et al. 2006, ZAMORA et al. 2012), as well as partial dissociation of casein as a result of high-pressure homogenization (SANDRA and DALGLEISCH 2005), were probably the reason for a weaker (as compared to non-homogenized milk) effect of the applied reagents on the parameters under analyses. This was reflected in the values of parameters describing the size of particles measured in homogenized milk samples, particularly at 100 MPa (in most cases, the effect of applied reagents was statistically insignificant at $\alpha = 0.01$) (Table 1). Particles measured in homogenized milk probably include more fat globules than non-homogenized milk, and consequently, the addition of solutions dissociating casein micelles has a lower effect on the values of the particle sizes obtained as a result of measurements compared to non-homogenized milk. This was also proven by the effect of their addition on an increase of d_v10 particles in non-homogenized milk (which was 3.5-2.2-fold) while for milk homogenized at 20 MPa and 100 MPa this increase was 1.9-1.3-fold and maximum of 1.4-fold, respectively (Figure 2*a*). Lower differentiation was found for d_v50 dimensions of milk, especially homogenized at 100 MPa, under analysis depending on their preparation for measurement (Figure 2*b*).

Changes in values of d_{43} for fat globules or their aggregates as a result of the addition of SDS and a combined addition of EDTA and Tween 20 would indicate fat globule flocculation in milk homogenized at 100 MPa intended for measurements without an addition of chemical reagents (Table 1). For milk homogenized at 100 MPa, this was confirmed by d_v90 values for the measured particles, which were reduced as a result of all applied reagents (Figure 2*c*). The addition of casein micelles dissociating reagents has an effect on particle size measurement and results in homogenized products due to a modified fat globule membrane, which contains casein through which globules can aggregate and form clusters or chains (LE THU et al. 2006, OLSON et al. 2004]. The effect on the analysed determinants of fat globules dispersion in homogenized milk could result from the dissociation of non-absorbed casein micelles, but also submicelles adsorbed at the surface of fat globules, thus causing disintegration of fat globule agglomerates.

The application of EDTA alone and a combination of SDS with trisodium citrate for milk sample preparation brought similar results. The application

of SDS alone only slightly affected an changes in particle size determinants in comparison to corresponding samples of samples of milk and cream without any addition of reagents. This may suggest disintegration of agglomerates during the measurement in water as a dispersant (McCrae and Lepoetre 1996) or the absence of fat globule agglomerations. The results of the research described in this paper, confirmed by the results of microscopic observations, indicate that agglomeration of fat globules did not occur in the emulsions under analyses. The exception was milk subject to homogenization, particularly at 100 MPa and cream with 20% fat content.

On account of the comparable efficiency of the applied reagents in dissociating micellar casein and deagglomeration of fat globules and a reduction or prevention of their agglomeration and maintaining the form of individual globules, particularly in cream or in homogenized milk, they can be applied interchangeably as long as the obtained results are controlled on an ongoing basis. Traditional microscopic observations are still applied in fat globule size examinations, often providing optimization of the particle size measurement results obtained by instrumental methods.

Conclusions

The method of preparing milk and cream for measurements using the instrumental method with laser diffraction analysis determines the fat globule size results to an extent depending on the fat content in the analysed sample and the method of its processing (e.g. homogenization). Dissociation of casein micelles performed before particle size measurements is of high importance for evaluating the size of fat globules in non-homogenized milk in view of the significant share of casein in its composition. With an increase in the fat content and in the size of fat globules, elimination of the non-fat components in cream intended for evaluation of fat globule sizes is gradually losing its plausibility. In turn, dissociation of casein micelles in homogenized milk takes on increased significance along with an increase of the pressure parameter of the process in terms of its effect on determinants of fat globule dispersion. With regard to the use of reagents for disintegrating fat globule agglomerates, it was found that for the emulsions under analysis, in the vast majority of cases, they did not contribute to any modification of the size of fat globules or their agglomerates, except for cream with the 20% fat content and milk homogenized at 100 MPa. Trends of changes of the analysed values characterizing fat globules of milk and cream and occurrence (or absence) of forms of emulsion instability, determined on the basis of measurement results, were confirmed by the results of microscopic observations conducted before adding

reagents. While evaluating the size of fat globules, full information can be obtained by using a two-stage procedure which includes both approaches, i.e. the microscopic method helping determine the occurrence of flocculation and the instrumental method, while in the absence of fat globule agglomerates it seems unnecessary to add reagents affecting their disaggregation.

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FATTY ACID PROFILE AND *TRANS* FATTY ACIDS CONTENT IN CEREALS AND CEREAL BARS FROM POLISH MARKET

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K e y w o r d s: lipid content, fatty acid profile, *trans* fatty acids, cereals, cereal bars.

A b s t r a c t

This study was aimed at determining lipid content, fatty acid composition and *trans* isomers content in fat extracted from cereals and cereal bars. Cereals and cereal bars were analyzed by gas chromatography. Analyses showed that they were characterized by a diversified content of fat and composition of particular groups of fatty acids (saturated SFA, monounsaturated MUFA and polyunsaturated PUFA). Only oat flakes turned out to be a good source of PUFA (38.83% of total fatty acids). The remaining products contained more SFA (mean: 45.12% and 47.73% in cereals and 63.31% in cereal bars) than PUFA (mean: 12.24% and 16.73% in cereals and 7.83% in cereal bars). Lipid of all examined products contained *trans* isomers of C18:1 and C18:2 acids. In lipids of cereals, the total content of these isomers did not exceed 0.5% of the total fatty acids. A higher content of these isomers was found in cereal bars (0.45–3.15%).

PROFIL KWASÓW TŁUSZCZOWYCH ORAZ ZAWARTOŚĆ IZOMERÓW *TRANS* W PŁATKACH I BATONIKACH ZBOŻOWYCH DOSTĘPNYCH NA POLSKIM RYNKU

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S ł o w a k l u c z o w e: zawartość lipidów, profil kwasów tłuszczowych, *trans* kwasy tłuszczowe, płatki, batoniki zbożowe.

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Abstrakt

Celem badań było określenie zawartości lipidów oraz składu kwasów tłuszczowych i zawartości izomerów *trans* kwasów tłuszczowych w płatkach i batonikach zbożowych. Płatki i batoniki zbożowe analizowano metodą chromatografii gazowej.

W badaniach wykazano, że badane produkty charakteryzowały się zróżnicowaną zawartością lipidów i zróżnicowanym składem poszczególnych grup kwasów tłuszczowych (nasyconych SFA, monoenowych MUFA i polienowych PUFA). Tylko płatki owsiane okazały się dobrym źródłem PUFA (38,83% w ogólnym składzie kwasów tłuszczowych). Pozostałe produkty zawierały więcej SFA (średnio: 45,12% i 47,73% w płatkach i 63,31% w batonikach zbożowych) niż PUFA (średnio: 12,24% i 16,73% płatki i 7,83% batoniki zbożowe). W lipidach wszystkich badanych produktów stwierdzono zawartość izomerów *trans* kwasu C18:1 i kwasu C18:2. W lipidach płatków całkowita zawartość tych izomerów nie przekracza 0,5% ogólnego składu kwasów tłuszczowych. Wyższe zawartości tych izomerów stwierdzono w batonikach zbożowych (0,45–3,15%).

Introduction

Cereals and cereal products are staple foods. Among cereal products the highest consumption is observed for breads, pasta, groats, flour, confectionery products and cereals. These products provide the body with necessary components such as fiber, vitamins, minerals and others. Fat present in man's diet is the main source of energy in a daily food ration, and also of essential fatty acids and fat-soluble vitamins. Its quality is largely determined by its fatty acid composition. Fatty acids present in high-fat foods have different effects on the body. Some saturated fatty acids (lauric (C12:0), myristic (C14:0) and palmitic (C16:0)) are detrimental to health as they increase both the low density lipoprotein (LDL) and high-density lipoprotein (HDL)- cholesterol and increase the LDL/HDL ratio. Of these three fatty acids, myristic acid appears to have the greatest impact (MENSINK and KATAN 1992, MENSINK et al. 2003, ZOCK et al. 1994). In turn, stearic acid does not increase the level of total cholesterol or LDL-cholesterol (WILLIAMS 2000). Polyunsaturated fatty acids are not synthesized by humans and need to be supplied with food. They perform a number of positive functions in the human body (GEBAUER et al. 2006, DE FILIPPIS et al. 2010). Some fatty acids with *trans* configuration have adverse effect on blood lipids and thereby increase the risk of coronary heart disease (ASCHERIO et al. 1999, STENDER and DYERBERG 2004, MOZAFFARIAN et al. 2006, 2009, DHAKA et al. 2011, KARBOWSKA and KOCHAN 2011). *Trans* fatty acids are found in fat of the ruminants (dairy products, beef, lamb) as a result of bacterial action in the rumen, and in margarines, shortenings, cooking fats and various foodstuffs produced with partially-hydrogenated oils. Their content in industrially-hydrogenated fats varies widely and may exceed 50% of the fatty acid content (BAYARD and WOLFF 1995, DANIEWSKI et al. 1997, 1998, ŻEGARSKA et al. 2000, PASZCZYK and ŁUCZYŃSKA 2013). Smaller amounts

of *trans* fatty acids occur naturally in ruminant fats. Another important sources of *trans* fatty acids in our diet include "hidden fats" contained in various food products. Literature data show also fast-food and snack products (DANIEWSKI et al. 1998, WAGNER et al. 2000), as well as some biscuits, chips and cakes (DANIEWSKI et al. 1997, 1998, 2000, ARO et al. 1998, DAGLIOGLU et al. 2000, 2002, ŻEGARSKA and BOREJSZO, 2002, MARTIN et al. 2005, PASZCZYK et al. 2007) to be rich sources of *trans* isomers in our diet. Investigations carried out in different countries demonstrated a high amount of *trans* isomers in some breakfast cereals and muesli (ARO et al. 1998, DAGLIOGLU et al. 2002, MAHESAR et al. 2010).

The market offers diversity of cereals and cereal bars. These products are convenient and easy to prepare. They are often eaten for breakfast. Considering that these products are very popular especially among children and young people, it is important to assess their quality. Hence, the purpose of this study was to evaluate the fatty acid composition, including the content of *trans* fatty acids, in fat extracted from cereals and cereal bars available on the Polish market.

Material and Methods

Material to be analyzed were cereals and cereal bars purchased in Poland. The cereals came from different producers and were categorized as: oat flakes produced by Sante, Raisio and Melvit (two different products of each producers were purchased), wheat and rice flakes with palm oil produced by Nestle, Dr Oetker and Lubella (two different products of each producers were purchased) and mixed cereals with the addition of milk, oil, cocoa, nuts or dried fruit produced by Nestle (four products), Melvit (two products), Crownfield (one product) and Lubella (one product). The cereal bars came from three producers: Nestle (ten different products), Crownfield (four products) and Sante (six products). Each sample was analyzed in duplicate and the results were reported as mean values.

Lipid content

Lipid content was determined using the Soxhlet's method. Dried samples were transferred to Soxhlet's apparatus and extracted for 4–9 h (6 overflow/1 h). To this end, the ether layer was distilled by means of an aggregate for distillation of solvents. Fat was dried at 105°C for 6–7 h to a constant weight, and then weighed.

The content of lipid [%] was calculated according to the formula:

$$x = \frac{[(b - a) \cdot 100]}{c}$$

where:
a – weight of flask [g]
b – weight of flask with extracted fat [g]
c – weight of sample [g].

Lipid extraction

Lipid extraction from the analyzed products was performed with the Folch's method (CHRISTIE 1973). To this end, the studied material was crushed (disintegrated) in a mortar and mixed. The sample (2 g) was homogenized (homogenizer IKA Ultra-Turrax T18 digital) for 1 min with 20 ml of methanol. Next 40 ml of chloroform were added and the process was continued for 2 min. The prepared mixture was filtered into a 250 ml glass cylinder. The solid residue was resuspended in 60 ml of a chloroform: methanol mixture (2:1 v/v) and homogenized again for 3 min. After filtration, the solid was washed with 40 ml of chloroform and 20 ml methanol. The combined filtrates were transferred to the same cylinder. Next, 0.88% sodium chloride in water (1/4 volume of filtrate) was added to the total filtrate, which was then shaken and left overnight. The upper layer was removed and a water : methanol mixture (1:1 v/v) was added to the lower layer, and the washing procedure was repeated. The remaining layer was filtrated by anhydrous sodium sulfate and distilled by means of an aggregate for distillation of solvents.

Preparation of fatty acid methyl esters

The fatty acids in the total lipids were esterified into methyl esters by saponification with 0.5N methanolic NaOH and transesterification with 14% BF_3 (v/v) in methanol (*Przygotowanie estrów*... PN-ISO 5509:2001).

Gas chromatography (GC) analysis

Separation of fatty acid methyl esters of the isolated fat was conducted with the gas chromatography (GC) method using a Hewlett Packard 6890 chromatograph with a flame-ionization detector (FID).

Determinations were carried out under the following conditions: capillary column – 100 m x 0.25 mm i.d. (Chromopack), film thickness – 0.20 μm, stationary phase – CP Sil 88, column temperature: 60°C (1 min) – 180°C, $\Delta t = 5°C\ min^{-1}$; injector and detector temperatures: 225 and 250°C, respectively; carrier gas: helium, flow rate: 1.5 ml min^{-1}, split 100:1.

Peaks of individual fatty acids were identified by comparing their retention times with those of methyl esters of reference fat with known fatty acids profile (BCR Reference Materials, symbol CRM 164). For identification of positional *trans* isomers of C18:1, use was made of the standards of methyl esters of those isomers (*trans* 6, *trans* 9, *trans* 11) (Sigma) and literature data. In turn, the *trans* isomers of C18:2 acid (*cis*, *trans* and *trans*, *cis*) were identified with the use of a mixture of standards of C18:2 isomers (Supelco) and literature data.

All samples were analyzed in duplicate and mean values were reported. The content of fatty acids was expressed as a percentage of the total fatty acids (wt %).

Statistical analysis

Significant differences in the profile of fatty acids in the cereal bars and cereals including oat flakes, wheat and rice flakes with palm oil and flakes mixed with the addition of milk, oil, cocoa, nuts or dried fruit were investigated using the analysis of variance ANOVA. Significant means were compared between the four groups by post-hoc Duncan's test at $\alpha = 0.05$ using STATISTICA 10. Data are presented as mean ± standard deviation (SD).

Results and Discussion

Differences in the content of lipid were observed both between and within the studied groups of cereals, with the largest ones found in mixed cereals with the addition of milk, oil, cocoa, nuts or dried fruits. The content of lipids in this group of products ranged from 6.0% to 16.8%. The oat flakes and wheat-rice flakes with the addition of palm oil contained between 5.40% and 7.70% and between 9.40% and 10.00% of lipids, respectively. Considerable differences in lipids content were also observed in the examined cereal bars, where it ranged from 7.20% to 17.90% (Table 1). For comparison, breakfast cereals analyzed in Pakistan by MAHESAR et al. (2010) had fat content from 23.6% to 26.1%. A lower lipids content was determined in corn chips (8.2%). In turn, according to ROE et al. (2013), the average content of lipids in breakfast cereals analyzed in the UK was 16.1% (11.6–20.5%), whereas in muesli and corn chips analyzed by DAGLIOGLU et al. (2002) in Turkey it was from 19.0% to 22.0%.

Lipid of the examined products was characterized by a diverse profile of fatty acids. The minimum and maximum contents of fatty acids and groups of fatty acids (saturated SFA, monounsaturated MUFA and polyunsaturated PUFA) are presented in Table 1. Table 2 shows mean values and standard deviation of fatty acids and sum of fatty acids.

Table 1
Range of fatty acid and *trans* fatty acid content in lipid of the analyzed products (% of total fatty acids)

Fatty acids	Cereals			Cereal bars (n = 20) min.–max.
	oat flakes (n = 6) min.–max.	wheat and rice flakes with the addition of palm oil (n = 6) min.–max.	mixed cereals with the addition of milk, oil, cocoa, nuts or dried fruit (n = 8) min.–max.	
Lipid content (g/100 g)	**5.40–7.70**	**9.40–10.00**	**6.00–16.80**	**7.20–17.90**
C6:0	0.01	0.01–0.21	0.01–0.13	0.01–0.13
C8:0	0.01–0.02	0.01–0.02	0.02–1.99	0.47–1.13
C10:0	0.02–0.04	0.01–0.03	0.02–1.52	0.05–1.88
C12:0	0.06–0.25	0.02–0.02	0.15–9.51	0.27–37.7
C14:0	0.29–0.42	1.00–1.01	0.75–3.97	1.19–16.17
C15:0	0.01–0.03	0.01–0.05	0.04–0.19	0
C16:0	16.80–17.19	38.71–38.72	24.49–34.43	13.81–30.03
C17:0	0.04–0.06	0.10–0.11	0.07–0.27	0.06–0.17
C18:0	1.58–1.90	4.32–4.44	3.09–23.96	4.0–13.15
C19:0	0.01–0.02	0.02–0.03	0.01–0.02	0.01–0.05
C20:0	0.13–0.19	0.39–0.41	0.23–0.76	0.21–0.54
Σ **SFA**	**19.54–20.92**	**44.90–45.23**	**40.66–57.68**	**46.38–81.84**
C16:1	0.09–0.27	0.17–0.22	0.16–0.53	0.10–0.32
C17:1	0.01–0.03	0.01–0.03	0.03–0.06	0.01–0.04
C18:1 c9	37.59–41.38	40.62–40.71	30.98–34.72	9.38–36.64
C18:1 c11	1.17–1.46	1.11–1.17	0.81–1.29	0.50–1.19
C18:1 c12	0.01–0.02	0.02–0.03	0.01–0.03	0.02–0.12
C18:1 c13	0	0.02–0.06	0	0.01–0.07
C20:1	0.39–0.81	0.01–0.17	0.09–0.29	0.09–0.30
Σ **MUFA**	**39.9–43.48**	**42.15–42.38**	**32.54–36.52**	**10.40–38.15**
C18:2 c9c12	35.87–38.48	11.88–11.96	8.36–22.65	3.59–14.52
C18:3 c9c12c15	0.78–2.15	0.33–0.35	0.40–0.55	0.18–0.68
Σ **PUFA**	**36.65–40.29**	**12.21–12.31**	**8.86–23.05**	**3.94–14.97**
Σ *trans* C18:1	0.09–0.12	0.13–0.15	0.11–0.30	0.30–2.97
Σ *trans* C18:2	0.06–0.07	0.01–0.25	0.11–0.27	0.09–0.32
Σ ***trans***	**0.15–0.19**	**0.32–0.38**	**0.32–0.41**	**0.45–3.15**
Others	0.09–0.19	0.03–0.09	0.04–0.51	0.11–0.54

Table 2

Fatty acid composition and *trans* fatty acid content in lipid of the analyzed products (% of total fatty acids, mean ± SD)

Fatty acids	Cereals			Cereal bars (n = 20)
	oat flakes (n = 6)	wheat and rice flakes with the addition of palm oil (n = 6)	mixed cereals with the addition of milk, oil, cocoa, nuts or dried fruit (n = 8)	
Lipid content (g/100 g)	**6.48 ± 1.15^{b}**	**9.77 ± 1.15^{a,b}**	**12.20 ± 5.57^{a}**	**13.28 ± 3.28^{a}**
C6:0	0.01 ± 0.01^{b}	0.14 ± 0.12^{a}	0.11 ± 0.10^{a,b}	0.07 ± 0.04^{a,b}
C8:0	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.72 ± 1.10^{a}	0.75 ± 0.41^{a}
C10:0	0.03 ± 0.01^{b}	0.03 ± 0.00^{b}	0.62 ± 0.80^{a,b}	1.17 ± 0.62^{a}
C12:0	0.15 ± 0.09^{b}	0.23 ± 0.01^{b}	3.37 ± 5.32^{b}	22.41 ± 13.21^{a}
C14:0	0.38 ± 0.06^{b}	1.00 ± 0.01^{b}	2.12 ± 1.66^{b}	10.16 ± 5.42^{a}
C15:0	0.03 ± 0.02^{b,c}	0.05 ± 0.00^{a,b}	0.09 ± 0.08^{a}	0.00 ± 0.00^{c}
C16:0	17.28 ± 0.54^{c}	38.72 ± 0.01^{a}	29.49 ± 5.07^{a,b}	21.78 ± 8.14^{b,c}
C17:0	0.06 ± 0.02^{b}	0.10 ± 0.01^{a,b}	0.15 ± 0.11^{a}	0.10 ± 0.03^{a,b}
C18:0	1.80 ± 0.16^{b}	4.40 ± 0.07^{a,b}	10.59 ± 11.61^{a}	6.56 ± 3.52^{a,b}
C19:0	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.02^{a}
C20:0	0.17 ± 0.03^{b}	0.40 ± 0.01^{a}	0.46 ± 0.27^{a}	0.30 ± 0.10^{a,b}
Σ SFA	**19.95 ± 0.65^{c}**	**45.12 ± 0.19^{b}**	**47.73 ± 8.87^{a,b}**	**63.31 ± 13.08^{a}**
C16:1	0.20 ± 0.08^{ka}	0.19 ± 0.03^{a}	0.29 ± 0.21^{a}	0.19 ± 0.08^{a}
C17:1	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}
C18:1 c9	38.69 ± 1.81^{a}	40.65 ± 0.05^{a}	33.36 ± 2.07^{a,b}	26.17 ± 10.50^{b}
C18:1 c11	1.32 ± 0.12^{a}	1.13 ± 0.03^{a,b}	1.05 ± 0.24^{a,b}	0.84 ± 0.28^{b}
C18:1 c12	0.02 ± 0.01^{b}	0.03 ± 0.01^{a,b}	0.02 ± 0.01^{b}	0.06 ± 0.03^{a}
C18:1 c13	0.00 ± 0.00^{c}	0.03 ± 0.02^{a,b}	0.01 ± 0.02^{b,c}	0.05 ± 0.02^{a}
C20:1	0.68 ± 0.20^{a}	0.17 ± 0.00^{b}	0.20 ± 0.10^{b}	0.14 ± 0.06^{b}
Σ MUFA	**40.93 ± 1.93^{a}**	**42.23 ± 0.13^{a}**	**34.97 ± 2.13^{a,b}**	**27.47 ± 10.82^{b}**
C18:2 c9c12	37.45 ± 1.16^{a}	11.91 ± 0.05^{b,c}	16.24 ± 7.26^{b}	7.45 ± 3.57^{c}
C18:3 c9c12c15	1.37 ± 0.57^{a}	0.34 ± 0.01^{b}	0.48 ± 0.08^{b}	0.37 ± 0.21^{b}
Σ PUFA	**38.83 ± 1.60^{a}**	**12.24 ± 0.06^{b,c}**	**16.73 ± 7.22^{b}**	**7.83 ± 3.63^{c}**
Σ *trans* C18:1	0.11 ± 0.01^{a}	0.14 ± 0.01^{a}	0.17 ± 0.11^{a}	0.93 ± 0.98^{a}
Σ *trans* C18:2	0.07 ± 0.01^{b}	0.22 ± 0.05^{a}	0.20 ± 0.08^{a}	0.17 ± 0.07^{a}
Σ *trans*	**0.17 ± 0.02^{a}**	**0.36 ± 0.03^{a}**	0.37 ± **0.05^{a}**	**1.10 ± 0.98^{a}**
Others	0.12 ± 0.05^{a}	0.05 ± 0.03^{a}	0.20 ± 0.27^{a}	0.30 ± 0.18^{a}

The values marked in the rows with the same letter are not significantly different ($P > 0.05$); a, b – significantly different ($P \leq 0.05$).

The total SFA in the analyzed oat flakes varied from 19.54% to 20.92%. The major fatty acid of this group was palmitic acid (16.80–17.19%). The oat flakes were characterized by a high percentage content of PUFA (36.65–40.29%), with predominant linoleic acid (35.87–38.48%). In the case of wheat-rice flakes and mixed cereals, the content of SFA was also different. The mean content

of SFA in wheat-rice flakes was significantly higher (45.12%) than in oat flakes (19.95%) and significant lower than in cereal bars (63.31%; $p \leq 0.05$). The content of SFA in wheat-rice flakes was from 44.90% to 45.23%, whereas in mixed cereals it was from 40.66% to 57.68%. Its mean value (47.73%) in mixed cereals was significantly higher than in oat flakes ($p \leq 0.05$). SFAs in wheat-rice flakes and mixed cereals were represented by palmitic and stearic acids, the content of which varied from 38.71% to 38.72 and from 4.34% to 4.44%, respectively (Table 1). In the case of mixed cereals, contents of these acids ranged from 24.49% to 34.43% and from 3.09% to 23.96%, respectively. The mean content of MUFA in wheat-rice flakes (42.23%) was significantly higher than in cereal bars ($p \leq 0.05$). The percentage content of MUFA in wheat-rice flakes was 42.15–42.38%, whereas in mixed cereals it was 32.54–36.52%. The wheat-rice flakes were characterized by a significantly lower content of PUFA than oat flakes (Table 2). The mean content of PUFA in wheat-rice flakes did not differ significantly from that in mixed cereals and cereal bars ($p > 0.05$). The PUFAs in wheat-rice flakes (12.21–12.31%) and mixed cereals (8.86–23.05%) were represented mainly by linolenoic acid (C18:2 *c*9, *c*12).

The mean content of SFA in cereal bars (63.31%) was significantly higher than in oat flakes and wheat-rice flakes ($p \leq 0.05$). The analyzed cereal bars were characterized by diversified contents of individual saturated fatty acids. Only a few cereal bars had a high content of palmitic acid (13.81–30.03%). Some of them contained more lauric and myristic acids. The high level of lauric acid (0.27% to 37.70%) and myristic acid (1.19% to 16.17%) in fat of some cereal bars can indicate coconut oil addition to these products as coconut oil is known to be a rich source of these acids (46.458% and 20.572%, respectively) (DAUQAN et al. 2011). As shown by literature data, their high intake may contribute to development of cardiovascular disease (ZOCK et al. 1994, WILLIAMS 2000). Furthermore, cereal bars contained from 10.40% to 38.15% of MUFA and from 3.94% to 14.97% of PUFA. The predominant MUFA was oleic acid C18:1 *c*9 (9.38–36.64%), whereas the main PUFA was linoleic acid C18:2 *c*9, *c*12 (3.59–14.52%). The percentage content of SFA in the examined products was higher than that of MUFA and PUFA (with the exception of oat flakes). Similar regularity was found by other authors. For example, muesli studied by DAGLIOGLU et al. (2002) contained 32.9% of SFA, 59.4% of MUFA and 7.7% of PUFA. Cereal bars studied by DERAWIAKA and GÓRSKA (2012) had from 46.0% to 73.8% of SFA, from 21.7% to 37.7% of MUFA and from 4.4% to 16.4% of PUFA. According to DAGLIOGLU et al. (2002), the content of SFA, MUFA and PUFA in corn chips was 43.5%, 42.4% and 14.1%, respectively. Finally, ARO et al. (1998) showed that muesli contained more SFA (50.77%) than cereals with vegetable oil (10.71%).

Lipid of all examined products was demonstrated to contain *trans* isomers of C18:1 and C18:2 acids. The higher content of those isomers was found in majority of cereal bars. The percentage content of C18:1 *trans* isomers in cereal bars was between 0.30% and 2.97%. The mean value of these isomers was 0.93% and did not differ significantly from the mean content of C18:1 *trans* isomers in oat flakes. A lower content of *trans* isomers in cereal bars was found by DEREWIAKA and GÓRSKA (2012). These authors found fatty acid *trans* isomers only in muesli bars with milk chocolate (0.20%).

Among the studied flakes, a higher content of C18:1 *trans* isomers was found in mixed flakes (0.11–0.30%), whereas a lower one in oat flakes (0.09–0.12%). *Trans* isomers of C18:2 acid in cereal bars, wheat-rice flakes and mixed cereals were at a similar level. A significantly lower content of these isomers was found in oat flakes ($p \leq 0.05$). The total content of *trans* isomers in the analyzed cereals was 0.17% in the oat flakes, 0.36% in wheat and rice flakes with the addition of palm oil and 0.37% in mixed cereals (Table 2).

ARO et al. (1998) found that cereals with vegetable oil were characterized by a higher content of total *trans* fatty acids (23.67%) than muesli (18.91%). In turn, muesli examined by DAGLIOGLU et al. (2002) contained 27.0% of *trans* isomers, whereas corn chips – 0.7% of total fatty acids. *Trans* fatty acids in Austrian breakfast cereals were at 0.21% (WAGNER et al. 2008), whereas breakfast cereals from the UK analyzed by Roe et al. (2013) contained 0.05 g/100 g FAME (range from 0.04 to 0.06 g/100 g FAME). In Pakistan cereal-based foods examined by MAHESAR et al. (2010) the content of *trans* fatty acids was varied. These authors reported that the content of *trans* fatty acids determined by GC ranged from 14.4 to 15.7 g/100 g of lipids in breakfast cereals, and was at 2.3 g/100 g of lipids in corn chips (one sample). In turn, a wide range of TFA values was found by AKMAR et al. (2013) in Malaysian breakfast cereals, i.e. from 1.57 to 4.82 g/100 g lipids (the highest in corn cereals) as well as in cereal beverages, i.e. from 4.74 to 6.60 g/100 g of lipids.

Conclusions

Cereals and cereal bars were characterized by diversified contents of lipid and composition of particular groups of fatty acids (saturated SFA, monounsaturated MUFA and polyunsaturated PUFA). Our studies showed that only oat flakes were a good source of PUFA. Other examined products contained more SFA than PUFA. Lipid of all the examined products contained *trans* isomers of C18:1 and C18:2 acids. In fat from the analyzed cereals the total content of these isomers did not exceed 0.5% of total fatty acid composition. Higher contents of these isomers were found in the analyzed cereal bars

(0.45–3.15%). Comparing the results with literature data, it can be concluded that the content of *trans* fatty acids in cereal products (cereals and cereal bars) were at the low level.

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SELECTED QUALITY INDICATORS OF POLISH REGIONAL DISH FROM WARMIA

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K e y w o r d s: Polish dumplings, Polish regional food, nutritional characteristics, organoleptic assessment.

A b s t r a c t

The objective of this study was to determine selected quality indicators and to evaluate the acceptability of traditional dishes from the Region of Warmia on the example of *dzyndzałki z hreczką i skrzeczkami* (dumplings stuffed with buckwheat topped with sour cream and bacon).

The nutritional value of the analyzed dish was determined from the recipe. The index of nutritional quality (INQ) was calculated based on an empirical formula. To determine the correct balance of an exemplary daily meal, reference GDA values were used to supplement the meal with an additional dish – chłodnik. The dish was prepared three times and evaluated by flavor profiling. The dish was analyzed to determine its sodium chloride content, DPPH· radical scavenging activity and inhibition of synthetic LDL cholesterol oxidation.

Dumplings have a high energy content of 305 kcal per 100 g serving, which provides 8.5 g of protein, 15.8 g of fat, 78 mg of cholesterol and nearly 35 g of carbohydrates. The dish is a very good source of sodium, but not enough calcium, vitamins A and C.The INQ values of the remaining minerals and vitamins were determined in the range of 0.5–0.7. The dish is characterized by a low antioxidant capacity (12.9%) and it is not capable of inhibiting the oxidation of synthetic LDL cholesterol ($C_{50} < 1$). Dumplings received high scores in a sensory evaluation. The dish combined distinctive sensory attributes characteristic of its ingredients, in particular spices, and significant differences in quality indicators were observed between the aroma and taste of marjoram and fat. Atypical, foreign and pungent aromas or tastes were not detected.

The results of this study indicate that regional dishes prepared with the use of locally available ingredients and traditional recipes can be a valuable component of the contemporary diet.

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WYBRANE WSKAŹNIKI JAKOŚCI POLSKIEJ POTRAWY REGIONALNEJ Z WARMII

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S ł o w a k l u c z o w e: polskie pierogi, polska żywność regionalna, charakterystyka żywieniowa, ocena organoleptyczna.

A b s t r a k t

Celem pracy była charakterystyka wybranych wskaźników jakości oraz ocena akceptowalności sensorycznej potrawy regionalnej z Warmii – *dzyndzałków z hreczką i skrzeczkami* (pierogi z farszem z kaszą gryczaną polane śmietaną i skwarkami).

Na podstawie składu recepturowego oszacowano wartość odżywczą potrawy oraz obliczono wskaźnik jakości żywieniowej INQ. W celu ustalenia prawidłowego zbilansowania przykładowego dziennego posiłku posłużono się referencyjnymi wartościami GDA, uzupełniając posiłek dodatkowym daniem – chłodnikiem. Potrawę wykonano trzykrotnie i oceniono sensorycznie metodą profilowania. Każdorazowo oznaczono zawartość chlorku sodu oraz zdolność wygaszania rodnika DPPH· i hamowania reakcji utleniania syntetycznego cholesterolu LDL.

Dzyndzałki charakteryzują się wysoką wartością energetyczną – 305 kcal, 100 g gotowej potrawy dostarcza 8,5 g białka, 15,8 g tłuszczu, 78 mg cholesterolu i około 35 g węglowodanów. Są bardzo dobrym źródłem sodu, natomiast niewystarczającym źródłem wapnia, witaminy A i C. Wartość INQ pozostałych składników mineralnych oraz witaminy E wynosi od 0,5–0,7. Potrawa cechuje się słabymi zdolnościami antyoksydacyjnymi przejawiającymi się zdolnością wygaszania rodnika DPPH· na poziomie 12,9% (EC_{50} = 947,7 mg). Wykazano także brak zdolności hamowania reakcji utleniania syntetycznego cholesterolu LDL (C_{50} < 1).

Oceniający uznali badaną potrawę za atrakcyjną pod względem sensorycznym. Cechowała się swoistymi wyróżnikami charakterystycznymi dla użytych surowców, szczególnie przypraw, a istotne statystycznie zróżnicowanie wyróżników zaobserwowano między zapachem i smakiem majeranku oraz tłuszczowym. Nie stwierdzono występowania nietypowych, obcych, ostrych zapachów i smaków.

Wyniki badań wskazują, że potrawy regionalne przygotowane z lokalnych surowców na bazie tradycyjnej receptury mogą być podstawą dobrze zbilansowanej współczesnej diety.

Introduction

The popularity of traditional and regional foods has soared in recent years. Numerous cook books, historical books and memoirs dedicated to regional cuisine have been published. Despite the above, there is a general scarcity of reliable and factual information confirming the geographic origins of a given product or dish. Scientific publications investigating regional cuisine are even less frequent. European research papers authored by French, Italian and German scientists (TRICHOPOULOU et al. 2007, KÖGL and TIETZE 2010) present the results of sensory evaluations, analyses of the fatty acid profile, selected health benefits, risks and consumer preferences associated with regional foods. Numerous review papers (WEICHSELBAUM et al. 2009, BOROWSKI 2010) discuss

the history, production technology and nutritional characteristics of traditional and regional foods in Europe. Despite the recent rise in the popularity of traditional cuisine, the knowledge about regional foods remains quite limited in Poland. The popularization of knowledge about the culinary heritage of European regions could contribute to the elimination of stereotypes and prejudices, it could strengthen international relations, provide an additional source of income for regions, including through tourism promotion. Promotional measures improve consumer awareness of a product and its region of origin, thus increasing product's competitive advantage on the international market. Research on traditional cuisine can contribute to the effectiveness of the socio-economic development strategy of the Region of Warmia and Mazury until 2020, whose priority goal is to increase the competitiveness of the local economy.

The objective of this study was to determine selected quality indicators and to evaluate the sensory acceptability of traditional dishes from the Region of Warmia on the example of *dzyndzałki z hreczką i skrzeczkami*. Our aim was to verify the research hypothesis that despite natural modifications in the composition and properties of original food ingredients over the years, the nutritional value of regional dishes supports rational eating habits and contributes to a well-balanced diet of contemporary consumers. Contemporary quality evaluation methods can be applied in analyses of regional and traditional foods to verify their quality and facilitate the registration of regional specialties with a protected designation of origin.

Material and Methods

The experimental material was *dzyndzałki z hreczką i skrzeczkami* a dish unique to the Region of Warmia, prepared based on a traditional recipe (ORŁOWSKA 2011) with the use of organically produced and locally supplied ingredients. The dish was composed of the following ingredients: raw bacon, 200 g; garlic, 8 g; salt, 15 g; wheat flour, 500 g; whole eggs, 180 g; rapeseed oil, 25 g; buckwheat groats, 200 g; smoked bacon, 150 g; onions, 200 g. 1.5 kg of dumplings were obtained.

The nutritional value of a 100 g portion was determined in view of recipe ingredients based on Food Composition and Nutrition Tables (KUNACHOWICZ et al. 2005). The index of nutritional quality (INQ), a measure of nutrient density, was calculated based on an empirical formula (GAWĘCKI 2012):

$$\text{INQ} = \frac{\text{amount of nutrient in 100 g of the product} \cdot \text{average energy requirement}}{\text{energy value of 100 g of the product} \cdot \text{reference intake for nutrient}}$$

Guideline daily amounts (GDA) (ANIOŁA 2011), which define the percentage of nutrients and energy per one serving of the product relative to the recommended daily nutrient and energy requirements, were used to plan a balanced meal. The dish was prepared three times, and it was evaluated by a team of 10 sensory panelists trained according to standard *Sensory analysis...* PN-EN ISO 8586:2014-03. The intensity of every analyzed attribute was evaluated by the flavor profiling method (*Sensory analysis...* PN-EN ISO 6564:1985) on a 6-point scale.

Numbers from 1 to 6 are assigned the following wordings referring sensibility of the discriminant:

1 – Non-detectable;
2 – Hardly detectable;
3 – Slightly detectable;
4 – Moderately detectable;
5 – Distinctively detectable;
6 – Strongly detectable.

Each dish was analyzed to determine its sodium chloride content by the Mohr method (*Wyroby garmażeryjne...* PN-A 82100:1985), DPPH: radical scavenging activity according to the method proposed by BRAND-WILLIAMS et al. (1995) and modified by SÁNCHEZ-MORENO et al. (1998) and MIELNIK et al. (2006), and inhibition of synthetic LDL cholesterol (SIGMA-ALDRICH) oxidation based on the method developed by ANDREASEN et al. (2001). Laboratory samples of approximately 200 g each were twice ground in the Stalgast 721129 meat grinder with a plate hole size of 3 mm. Ground samples were thoroughly mixed and homogenized. The number of replications was 3 to 6, subject to the type of analysis. In order to estimate the antioxidant properties using the DPPH· method, 10 g were weighed with an accuracy of 0.01 g and 50 cm^3 of analytical grade methanol were added. The sample was then homogenised in a homogeniser (Universal Laboratory Aid, type MP W-309) for 30 s at 10,000 rpm. The sample was then centrifuged in a laboratory centrifuge, type WE-2, for 15 min at 3,000 rpm and filtered through a medium-grade filter (EUROCHEM BGD 12/5) to a graduated cylinder to read the volume of the supernatant. 1 cm^3 was collected from the prepared extract and dilutions in g cm^{-3} were prepared. From each of them, 0.1 g cm^{-3} was collected to a test tube and 3.9 cm^3 of a methanol DPPH· solution at a concentration of 0.025 g l^{-1} was added. The test tubes were tightly sealed with parafilm and left in a dark place at room temperature. Absorbance was measured at a wavelength of 515 nm using a UV/VIS spectrophotometer on an hourly basis until a constant value in relation to the reagent blank (i.e. methanol) was set.

DPPH· radical scavenging ability was determined based on a curve reflecting the relationship between the % of the residual DPPH· radical and the

amount of the sample. The equation of a straight line was interpreted as an EC_{50} ratio which determines the amount of mg of the tested raw material required to reduce the initial concentration of the synthetic DPPH· radical by 50% under the reaction conditions. The results were expressed in % using the formula below, and in relation to synthetic, water-soluble Trolox (an analog of vitamin E).

$$\% \text{ of the residual DPPH}\cdot = \frac{(\text{absorbance of the test sample}}{\text{absorbance of the control sample})} \cdot 100$$

Oxidation of the LDL fraction was determined based on spectrophotometric measurements at a wavelength of 234 nm. The reaction was induced by copper ions by adding 10 µl of 0.9 mM $CuSO_4$ dissolved in PBS (Cu concentration of 5 µM, temperature of 37°C, incubation for approx. 3 hours, pH = 7.4), according to a method by ESTERBAUER et al. (1989), with modifications by ANDREASEN et al. (2001). The antioxidant activity of the samples was measured as the ability to inhibit the formation of lipid dienes compared to the control sample. Synthetic LDL was diluted to a concentration of 0.2 mg ml^{-1} in a 0.01 M phosphate buffer (PBS). The extracts were tested at concentrations of 40–120 µl for 120 min. and the absorbance values were read every 10 and 20 min. The control sample of solvent/water was mixed in a 1:1 ratio.

The ability to inhibit the oxidation of LDL was estimated based on the absorbance-time curve equation and the inhibition percentage was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(C - S)}{C} \cdot 100$$

where:
C – maximum absorbance of the control sample
S – absorbance of the sample corresponding to the absorbance of control samples at the time the control sample exhibited maximum absorbance

If the value read on the spectrophotometer was higher than zero (positive), the sample exhibited antioxidant activity, i.e. it had the ability to inhibit cholesterol oxidation. In addition, the C_{50} factor was calculated based on the time required to obtain a 50% value of absorbance in test samples compared to the control sample.

$$C_{50} = \frac{t_{50} \text{ for the test sample}}{t_{50} \text{ for the control sample}}$$

If the value of the C_{50} factor was higher than one, the sample had both antioxidant properties and the ability to inhibit cholesterol oxidation.

The results were processed by Kruskal-Wallis ANOVA and Friedman's ANOVA in the Statistica 10.0 PL application. The data were not normally distributed (Shapiro-Wilk test) and did not meet the homogeneity of variance assumption (Levene's test of homogeneity of variance, Brown-Forsythe test, independent samples *t*-test at significance level of $\alpha = 0.05$), therefore, non-parametric tests for multiple independent samples were performed.

Results and Discussion

Selected quality indicators of dzyndzałki z hreczką i skrzeczkami

The main ingredients of *dzyndzałki z hreczką i skrzeczkami* are wheat flour, buckwheat groats, raw and smoked bacon, which are responsible for the product's relatively high energy value of 305 kcal per 100 g. The evaluated dish is characterized by a high content of carbohydrates and fat, including saturated and monounsaturated fatty acids. A 100 g portion provides 8.5 g of protein (Figure 1). The INQ value of protein (Figure 2) is higher than 1.0, which indicates that *dzyndzałki z hreczką i skrzeczkami* are well-balanced with regard to protein content, and they cover daily energy requirements. The dish is abundant in total fat (15.8 g/100 g) with a high INQ of 1.5, but it contains mostly saturated and monounsaturated fats whose levels should be kept to a minimum in a healthy diet.

Saturated fatty acids, which are found mainly in animal products (lard, fatback, butter, meat, cheese, eggs), can increase serum LDL cholesterol levels. High levels of unhealthy fatty acids contribute to the risk of colon, mammary gland and prostate cancer (CYBULSKA and KŁOSIEWICZ-LATOSZEK 2010, GAWĘCKI 2012). Polyunsaturated fatty acids (PUFAs), including essential fatty acids (n3/n-6 EFAs), deliver numerous health benefits. They delay cell ageing (FARZANEH-FAR et al. 2010), exert anticarcinogenic effects (AUGUSTSSON et al. 2003) and reduce blood triglyceride levels (DAVIDSON et al. 2007). The latest Polish dietary guidelines (*Normy żywienia...* 2012) no longer define the desired n-6/n-3 PUFA ratio, instead they set reference values for the consumption of EFAs and recommend a reduction or elimination of saturated fatty acids and trans fatty acids from the diet. In the referenced guidelines, adequate intake (AI) values have been defined for linoleic acid at 4% of total energy and alpha-linolenic acid at 0.5% of total energy.

Fat intake is associated with dietary cholesterol levels. Maximum intake values for dietary cholesterol are not defined by Polish dietary guidelines

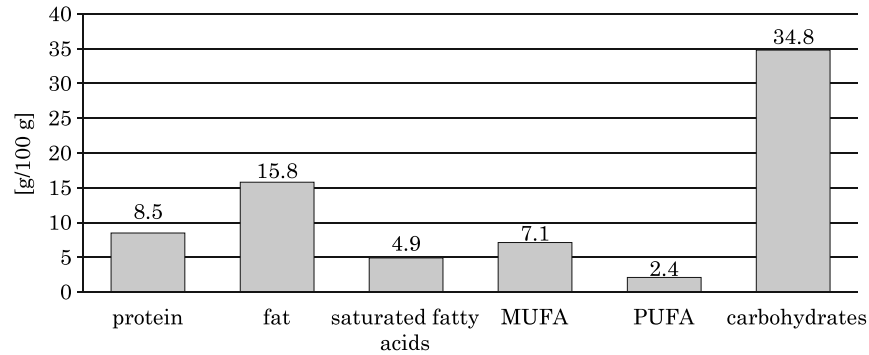

Fig. 1. The content of selected nutrients of *dzyndzałki z hreczką i skrzeczkami* [g/100 g of product]

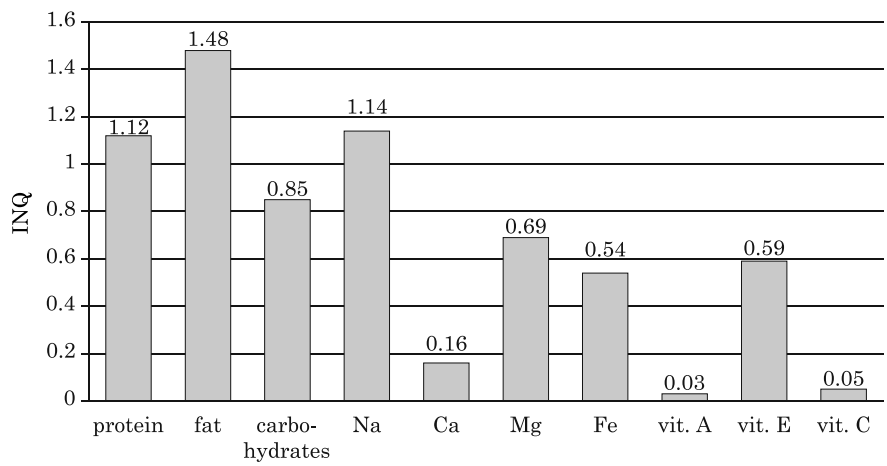

Fig. 2. Nutritional density of selected nutrients of *dzyndzałki z hreczką i skrzeczkami*

(*Normy żywienia...* 2012). *Dzyndzałki z hreczką i skrzeczkami* provides 78 mg of cholesterol per 100 g serving, mainly from egg yolks. Raw chicken eggs contain 391 mg of total cholesterol per 100 g (KUNACHOWICZ et al. 2005). Animal products are abundant in cholesterol, but the above does not imply that atherosclerosis and other pathological changes are directly induced by excessive consumption of dietary cholesterol.

Dzyndzałki z hreczką i skrzeczkami are a relatively well-balanced source of carbohydrates (INQ = 0.85, Figure 2). In line with nutritional recommendations, a higher percentage of total energy (55%) should be supplied by carbohydrates than fats. The main source of energy in the daily diet should be non-starch polysaccharides from cereals, legumes, root and tuber vegetables (GAWĘCKI 2012).

The INQ value of sodium in *dzyndzałki z hreczką i skrzeczkami* (Figure 2) is insignificantly higher than 1.0 despite the product's high sodium content (416 mg/100 g, Figure 3). The analyzed dish contains approximately 1 g of salt, which is within the reference values for salt set at 1% of the product's total weight. According to the latest Polish dietary guidelines, the daily sodium intake (AI) of adult consumers should not exceed 1500 mg (*Normy żywienia...* 2012).

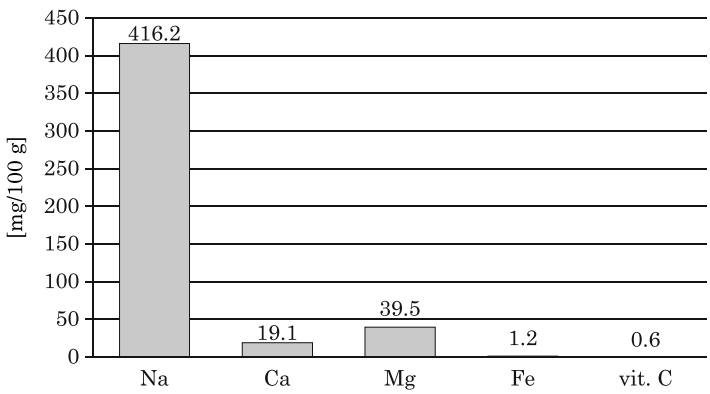

Fig. 3. The content of minerals and vitamin C in mg/100 g of *dzyndzałki z hreczką i skrzeczkami* [mg/100 g of product]

The amount of salt added to food increases sodium and chlorine levels in the body, which stimulates muscle and nerve cells and increases the permeability of biological membranes. In addition to spices, home-made foods also contain high amounts of added salt, which increases the daily intake of sodium chloride. According to the National Food and Nutrition Institute, the main sources of sodium, the major component of table salt, in the Polish diet are cereal products (22.5%), meat and meat products (19.4%), milk and dairy products (5.8%), vegetables and legumes (5.7%) (WOLNICKA and JAROSZ 2008).

Dzyndzałki z hreczką i skrzeczkami do not provide sufficient quantities of calcium (relative to the energy requirement), and the dish is not well-balanced with regard to calcium content. It does not contain calcium-rich ingredients such as ripened cheese, milk, fermented milks, kale or beans (GAWĘCKI 2012). Buckwheat groats are abundant in magnesium, and *dumplings from Warmia* are an ample source of that micronutrient (39.5 mg/100 g, Figure 3), but their magnesium content is insufficient relative to the energy requirement. The recipe for *dzyndzałki z hreczką i skrzeczkami* contains animal-derived ingredients, and the evaluated dish is rich in iron (Figure 3). Despite the above, the

INQ of iron was low at 0.54, which indicates that *dumplings* is not a good source of iron relative to the average requirement for energy provided by the dish (Figure 2). *Dzyndzałki z hreczką i skrzeczkami* is also deficient in vitamin A (Figure 2 and Figure 4), which is found mainly in fish oil, dairy products (butter, eggs) and vegetables, mostly carrots and leafy vegetables (GAWĘCKI 2012).

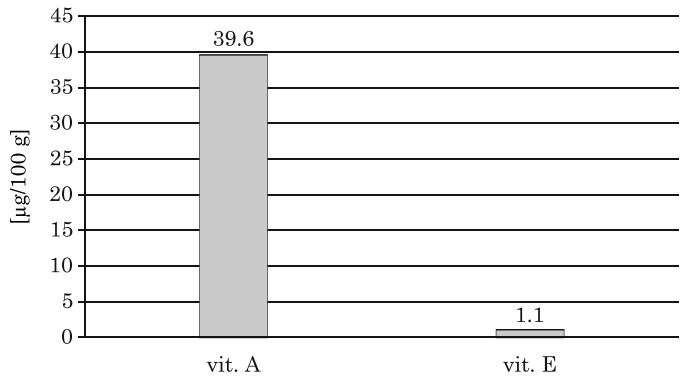

Fig. 4. The content of vitamins in µg/100 g of *dzyndzałki z hreczką i skrzeczkami*

Vegetable oils and margarine are rich in vitamin E, which is also found in selected animal products. Vitamin E is one of the major scavengers of superoxide radicals, and it protects lipids against oxidation (SROKA et al. 2005). A 100 g serving of *dzyndzałki z hreczką i skrzeczkami* supplies approximately 1 µg of vitamin E (Figure 4) with INQ < 1 (Figure 2), which indicates that the analyzed product is not a rich source of vitamin E relative to the energy requirement.

Cruciferous vegetables are rich in vitamin C, and foods containing those ingredients are a valuable source of ascorbic acid. Vitamin C plays an important biological role by reducing Fe^{3+} ions to Fe^{2+} ions, which improves iron absorption from the duodenum. Under certain conditions, vitamin C is capable of destroying animal cells, including cancer cells. Vitamin C is a potent antioxidant, and *in vitro* studies demonstrated that it protects blood lipid fractions (LDL) against oxidation. Vitamin C can also act as a pro-oxidant, but ascorbate doses that induce pro-oxidant effects have not been determined (SROKA et al. 2005). *Dumplings from Warmia* are not a good source of vitamin C whose concentrations are low relative to the energy requirement.

Regional foods can be a part of a healthy and well-balanced diet. A healthy meal incorporating traditional dishes was planned with the use of GDA reference values (Table 1). GDA values define the percent content of nutrients

and energy per serving relative to the recommended daily intake. They enable consumers to make well-informed choices and plan a well-balanced diet where GDA values do not exceed 100% (Figure 5).

Table 1

GDA values for the planned meal [%]

Dish	Energy value	Protein	Carbohydrates	Fat	Saturated fatty acids	Sodium	Salt
Chłodnik, 200 g	2.8	5.2	1.6	6.8	12.1	20.8	23.4
Dzyndzałki z hreczką i skrzeczkami, 250 g	38.0	42.5	32.3	56.5	62.0	43.3	41.8
Σ	40.8	47.7	33.9	63.3	74.1	64.1	65.2

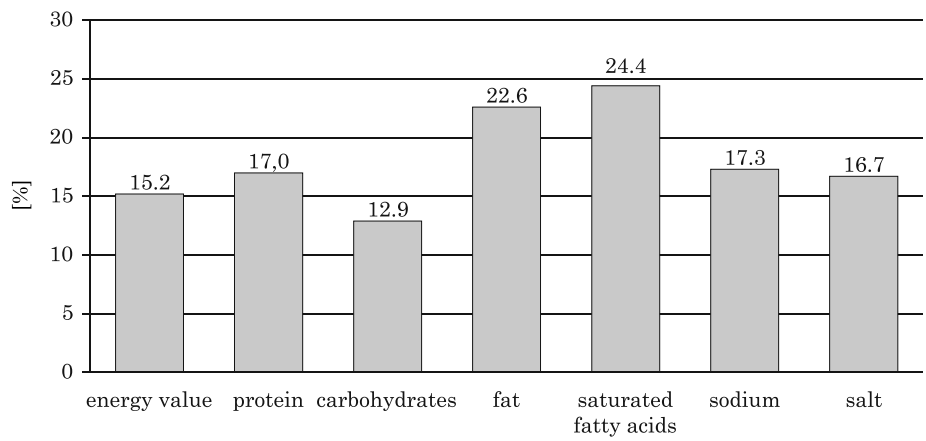

Fig. 5. The percentage of guideline daily amounts of energy and macronutrients of *dzyndzałki z hreczką i skrzeczkami*

The planned meal comprised two regional dishes – *chłodnik* (200 g) and *dzyndzałki z hreczką i skrzeczkami* (6 dumplings – approximately 250 g).

The planned meal indicates that regional products can constitute the basis of a healthy and well-balanced diet. GDA values provide consumers with simple information about products and ingredients that are missing from the diet. The planned meal (Table 1) should incorporate other products to keep sodium chloride levels within the recommended range. The intake of sodium, fat and, above all, saturated fatty acids, should be reduced.

The Mediterranean diet is an excellent example of healthy eating plan that incorporates traditional foods rich in macronutrients and micronutrients, in particular calcium and magnesium, and vitamins, including vitamin E (VASILOPOULOU and TRICHOPOULOU 2009). The main ingredients of the Greek diet are fresh vegetables (tomatoes, leeks), legumes, cereals, fresh fruit, eggs, feta-type cheese, yogurt and fish. Meat and meat products are consumed in limited quantities. Olive oil is an important ingredient in most dishes, and red wine intake is relatively high. The Mediterranean diet is well balanced, and it is characterized by average daily energy intake below 2473 kcal, where the following percentage of energy is provided by the following nutrient groups: protein – 12%, dietary fat – 40.3%, carbohydrates – 41.4% and dietary fiber – 2.4% (TRICHOPOULOU et al. 2006).

Antioxidant activity

In recent years, researchers and nutrition scientists have been emphasizing the beneficial effects of antioxidant compounds, mainly polyphenols (DRUŻYŃSKA and KLEPACKA 2005). Antioxidants inhibit the activity of enzymes that participate in carcinogenesis, and play an important role in preventing cardiovascular diseases and Alzheimer's disease (SZLACHTA and MAŁECKA 2008).

Dzyndzałki z hreczką i skrzeczkami contain processed cereal grains, mostly buckwheat groats, which are responsible for the antioxidant properties of the dish whose DPPH· radical scavenging activity was determined at 12.9% (EC_{50} = 947.7 mg – Table 2).

Table 2

Antioxidant activity of a selected dish measured by two different methods

Antioxidant properties of the dish whose DPPH· radical scavenging activity			
%	Equation of the standard curve	$EC_{50\ [mg]}$	μmole Trolox/g
12.9	*% of the residual DPPH·* = 99,079 – 9,758* sample concentration mg ml^{-1}	947.7	21.2
Antioxidant activity is associated with the inhibition of LDL cholesterol oxidation			
%	Equation of curve of absorbance – time	C_{50}	C_{50} Trolox
58.7	A = 0.4325 + 0.0002*time	0.32	6.3

Light and dark varieties of buckwheat groats are rich in flavonoids, rutin and isovitexin, but their content is reduced by nearly half during cooking. Wheat contains inositol hexaphosphate, which is resistant to heat processing,

but is degraded during seed germination and dough fermentation. Cereal germ is also a source of selenium and vitamin E (SZAJDEK and BOROWSKA 2004). ZIELIŃSKI and KOZŁOWSKA (2000) classified cereals in the following descending order based the antioxidant activity of their methanol seed extracts: buckwheat, barley, oats, wheat and rye. The synergistic interactions between tocopherols (α and γ fractions) and phospholipids are responsible for the antioxidant properties of vegetable oils. *Dzyndzałki z hreczką i skrzeczkami* were prepared with the use of rapeseed oil, a rich source of sinapinic, ferulic, caffeic and coumaric acids (SZAJDEK and BOROWSKA 2004).

Antioxidant activity is associated with the inhibition of LDL cholesterol oxidation because free radicals are responsible for lipid oxidation (DRUŻYŃSKA and KLEPACKA 2005). Lipids contained in meat products, including dietary cholesterol, are particularly susceptible to oxidation, and the content of cholesterol oxidation products (COPs) during food processing and storage can reach up to 10% of total cholesterol content (DEREWIAKA et al. 2008). Autooxidation products, including low-molecular-weight volatile compounds such as short-chain aldehydes, are responsible for the undesirable (rancid) odors and flavors in foods. They also deteriorate the color, texture, nutritional value and safety of meat products. Autoxidation products degrade essential fatty acids, which leads to the loss of their biological properties. Rancid fat also contributes to the degradation of biotin, riboflavin, ascorbic acid and pantothenic acid (HĘŚ and KORCZAK 2007).

The analyzed product's ability to inhibit the oxidation of synthetic LDL cholesterol was established based on literature, and it was confirmed by the results of the Trolox equivalent antioxidant capacity assay where the inhibitory rate was determined at 58.7% relative to Trolox (100%) and the C_{50} factor was determined at 6.3 for Trolox and 0.3 for *dzyndzałki z hreczką i skrzeczkami* (Table 2). The value of the C50 factor for the analyzed product (below 1.0) indicates that *dumplings* are practically unable to inhibit the oxidation of the LDL cholesterol fraction.

Sensory evaluation of dzyndzałki z hreczką i skrzeczkami

The null hypothesis (H_0, postulating an absence of statistically significant differences between quality indicators in each evaluation) was rejected in favor of the alternative hypothesis (H_1, postulating the presence of statistically significant differences between at least two quality indicators in each evaluation) based on the results of statistical analyses (H-test for *dzyndzałki z hreczką i skrzeczkami*, evaluation I – 94.53, evaluation II – 84.94, evaluation III – 95.98, $N = 150$) and estimated probability of $P < 0.001$. Multiple

comparisons of mean ranks for all samples were performed to determine differences in quality indicators in each evaluation. In *dzyndzałki z hreczką i skrzeczkami* (Figure 6), significant differences in quality indicators were observed between the taste and aroma of marjoram and fat.

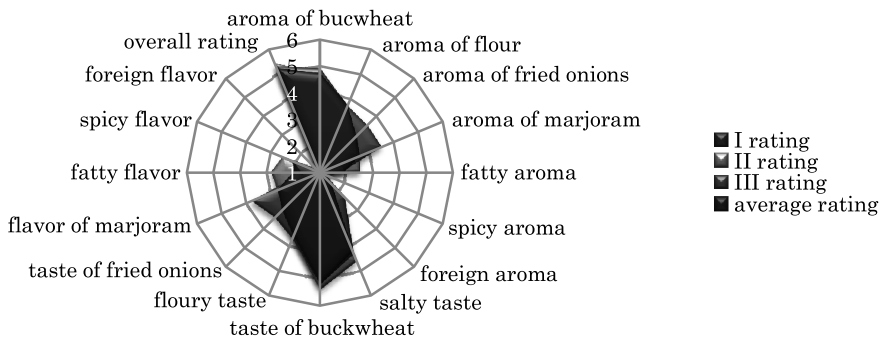

Fig. 6. Sensory profile of flavor and taste of *dzyndzałki z hreczką i skrzeczkami*

Dzyndzałki z hreczką i skrzeczkami received high scores in a sensory evaluation. The dish combined distinctive sensory attributes characteristic of its ingredients, in particular spices. The most perceptible smells and tastes were that of buckwheat (4.7 and 5.2 points, respectively), the smell of flour (3.7 points) and a salty taste (4.2 points). The overall rating was at a level of 5.2 points. Statistically significant quality factor differences were observed between the smell and taste of marjoram and of fat in particular ratings. Atypical, foreign and pungent aromas or tastes were not detected.

Conclusion

The results of this study indicate that regional dishes prepared with the use of locally available ingredients and traditional recipes can be a valuable component of the contemporary diet. The proposed lunch menu comprising *dzyndzałki z hreczką i skrzeczkami*, a local specialty of the Region of Warmia, was evaluated based on GDA reference values. The assessed product is characterized by a satisfactory nutritional value, it provides diverse nutrients, and therefore it can be successfully incorporated into a healthy and well-balanced diet.

The quality assessment method used in the study can be used in evaluations of traditional and regional foods. The sensory analysis revealed the distinctive attributes of *dzyndzałki z hreczką i skrzeczkami*. The evalu-

ated dish was characterized by a pleasing appearance, rich flavor and balanced, traditional taste. Atypical, foreign and pungent aromas or tastes were not detected.

Translated by Joanna Jensen

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REMOVAL OF LANDFILL LEACHATE TOXICITY BY ADSORPTION ON WHITE ROT FUNGI (*PLEUROTUS OSTREATUS*)*

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K e y w o r d s: landfill leachate, municipal solid waste, *Pleurotus ostreatus L.*, phytotoxicity test, *Sinapis alba* L.

A b s t r a c t

Municipal solid waste landfills are considered to be important sources of groundwater contamination due to the leakage of leachate. Landfill leachate is undoubtedly one of the most challenging wastewaters in terms of treatment. Fungi can be used to treat a landfill leachate. Therefore, the aims of this research were to evaluate the biosorption potential of *Pleurotus ostreatus* as low-cost adsorbent for the toxicity removal from raw landfill leachate. The objective was also to study the change of leachate toxicity before and after biosorption tests using *Sinapis alba* L. growth inhibition test. It can be concluded that the growth inhibition (%) of *Sinapis alba* L. for landfill leachate samples after biosorption tests were in the range of 31.55–96.16%. These samples were strongly toxic, but the toxicity compared to samples before biosorption tests decreased for all samples.

WYKORZYSTANIE BOCZNIAKA OSTRYGOWATEGO (*PLEUROTUS OSTREATUS*) DO REDUKCJI TOKSYCZNOŚCI ODCIEKÓW ZE SKŁADOWISKA ODPADÓW

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S ł o w a k l u c z o w e: odcieki ze śladowiska, odpady komunalne, *Pleurotus ostreatus L.*, testy fitotoksyczności, *Sinapis alba* L.

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Abstrakt

Składowiska odpadów komunalnych poprzez powstające odcieki mogą być istotnym źródłem skażenia wód gruntowych. Odcieki są najbardziej problematycznymi wodami odpadowymi, jeżeli chodzi o ich oczyszczanie. Grzyby mogą być wykorzystywane do oczyszczania odcieków w składowiskach.

Przeprowadzono badania, w których oceniono możliwości wykorzystania potencjału biosorbcyjnego *Pleurotus ostreatus* jako taniego adsorbentu wykorzystanego do redukcji toksyczności odcieków w składowiskach. Celem eksperymentu była ocena zmiany toksyczności odcieków przed zastosowaniem biosorbentu na roślinie testowej *Sinapis alba* L. i po jego użyciu.

Na podstawie przeprowadzonych badań można stwierdzić, że inhibicja wzrostu [%] *Sinapis alba* L. po zastosowaniu biosorbentu na odciekach wynosiła 31,55–96,16%. Badane odcieki były silnie toksyczne, jednak po zastosowaniu biosorbentu toksyczność znacząco zmalała dla wszystkich testowanych koncentracji.

Introduction

Landfills are one of those humans; activities that are changing the fate of the natural ecosystems (WONG et al. 2016a, KUMARI et al. 2016, KODA et al. 2013). Sanitary landfilling is a widely used large-scale waste disposal method worldwide, especially for municipal solid waste (MSW) (KODA et al. 2016, WONG et al. 2016b). MSW landfills are considered to be important sources of groundwater contamination due to the leakage of leachate, a complex mixture of pollutants having high chemical oxygen demand, high ammonium nitrogen content and lasting toxicological characteristics (GWOREK et al. 2016, HAN et al. 2016). Landfill leachate is the result of water percolating through waste deposits that have undergone aerobic and anaerobic microbial decomposition (MUKHERJEE et al. 2014). Its composition is a function of the type of waste in the landfill, landfill age, climate conditions, and hydrogeology of the landfill site (BRENNAN et. al. 2017). A landfill site will produce leachate throughout its working life and also for several hundred years after it is decommissioned. The control of a landfill site, and appropriate treatment of the leachate it produces, is paramount in the protection of the surrounding environment, as leachate contamination of groundwater, rivers, lakes and soils has the potential to negatively affect local habitats, resources and human health (BRENNAN et. al. 2017).

Landfill leachate may be characterized as a water-based solution consisting of four groups of contaminants: (1) dissolved organic matter, such as alcohols, acids, aldehydes, and short chain sugars; (2) inorganic macro components, which include common cations and anions (e.g., sulfate, chloride, iron, aluminum, zinc, and ammonia); (3) heavy metals (i.e., Pb, Ni, Cu, Hg, etc.); and (4) xenobiotic organic compounds such as halogenated organics (e.g., PCBs, dioxins, etc.) (Ren and YUAN 2015).

The conventional landfill leachate treatment includes physico-chemical treatments, and biological treatments. Physico-chemical treatments are usually used to reduce suspended solids, colloidal particles, color, and certain toxic compounds (REN and YUAN 2015). However, conventional treatment technologies become less effective and more expensive when situations involving high volumes and low metal concentrations are encountered (KAPOOR and VIRARAGHAVAN 1995, XIANGLIANG et al. 2005).

Biosorption, which involves the use of biomass or natural substances as sorbents, presents an attractive alternative to the traditional physicochemical means for removing toxic heavy metal from grounds and wastewaters (SAEED et al. 2005, GONG et al. 2005, DAVIS et al. 2003, HOLAN and VOLESKY 1995, XU and LIU 2008, PAVASANT et al. 2006, KOCAOBA and ARISOY 2011).

The uptake of heavy metals by biomass can take place by an active mode (dependent on the metabolic activity) known as bioaccumulation or by a passive mode (sorption and/or complexation) termed as biosorption (KAPOOR and VIRARAGHAVAN 1995). Biosorption, based on the metal binding capacities of various biological materials, has gained attention in the recent years due to high efficiency and low cost. Fungi, yeast, bacteria, algae have proven to be very effective in removing heavy metals from solutions and their biosorption behavior of heavy metals have been extensively studied (VASUDEVAN et al. 2002, XIANGLIANG et al. 2005, WANG 2002). In recent years, numerous studies have showed that some cultivated mushrooms can bioaccumulate considerable amount of metal ions. Recently, fungi, with their high tolerance and resistance to toxicity, have been recognized as an excellent candidate for treating leachate. Research has shown that white-rot fungi have developed nonspecific mechanisms to degrade an extremely diverse range of very persistent or toxic environmental pollutants (REN and YUAN 2015).

The aims of this research were to evaluate the biosorption potential of *Pleurotus ostreatus* (*P. ostreatus*). The potential of fungal biomass as low-cost adsorbent for the toxicity removal from raw leachate was determined. The objective was also to study the change of leachate toxicity before and after *P. ostreatus* biosorption using *Sinapis alba* L. growth inhibition test.

Materials and Methods

Leachate sampling

The municipal landfill row leachate employed in this study, was collected from the Kuchynky landfill, with capacity of 110 10^3 kg/day. The Kuchynky landfill is classified in the S-category for "other waste", sub-category S-OO3.

The area of the landfill is 70,700 m^2 in five stages, with a total volume of 907,000 m^3, i.e. around 1,000,000 10^3 kg of waste. The planned service life of the facility is up to 2018. The facility receives waste (in the category of "other waste") from a catchment area with a population of around 75,000 residents. The annually deposited amount of waste is around 40,000 10^3 kg, of which 50% is from the communal sphere (VOBĚRKOVÉ et al. 2017).

The experimental investigation was conducted in April 2015. Two samples (0.5 l/sample) of raw landfill leachate were collected in plastic bottles. The samples were packed in cool boxes (8–15°C) and were transported to the laboratory for analysis. Leachate samples were analyzed for pH, electrical conductivity (EC), chemical oxygen demand (COD) and a series of metals (Cd, Cr, Ni, Pb, Zn, Hg). Landfill leachate characteristics (years 2008–2015) are shown in Table 1.

Table 1

Characteristic of raw landfill leachate used in experiment

Parameter	Unit	Mean*
pH		8.303 ± 2.33
$N\text{-}NO_2^-$	mg l^{-1}	0.318 ± 0.61
$N\text{-}NO_3^-$	mg l^{-1}	5.546 ± 9.70
P	mg l^{-1}	4.908 ± 1.20
Cd	mg l^{-1}	0.018 ± 0.02
Hg	mg l	0.001 ± 0.00
AOX	µg l^{-1}	1027 ± 201.29
Zn	mg l^{-1}	0.289 ± 0.26
Cr	mg l^{-1}	0.940 ± 0.43
Pb	mg l^{-1}	0.036 ± 0.03
Ni	mg l^{-1}	0.343 ± 0.25
EC	mS m^{-1}	1073 ± 150.93
COD_{Mn}	mg l^{-1}	370 ± 95.25
As	mg l^{-1}	0.043 ± 0.02
PAU	µg l^{-1}	0.411 ± 0.21
N_{total}	mg l^{-1}	507.42 ± 156.65

* Results are the mean of values for the years 2008–2015 and ± indicate standard deviation.

Biosorption materials

Fresh fruiting bodies of *P. ostreatus* were obtained from a vegetable market, were washed with deionized water, dried at 60±1°C in laboratory dryer (Ecocell) for a period of 16 h, ground into powder using a mill (IKA® MF10 basic microfine grinder drive) and sieved through a 800 µm mesh sieve to obtain raw biomass for further use.

Biosorption experiments

The biosorption experiment was performed at 25°C in 250 ml flasks as follows: 40 g of *P. ostreatus* biosorbent was added to flasks containing 300 ml of raw leachate. The flasks were stirred with a magnetic stirrer at 150 rpm for 12 h. The biosorption tests were replicated twice. The mean values from the two duplicate were used. The leachate samples after the biosorption experiment were filtered and toxicity in the filtrate was determined using the *Sinapis alba* L. growth inhibition test. The scheme of the biosorption experiment is shown in Figure 1.

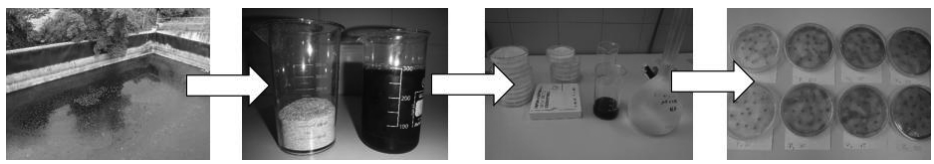


Fig. 1. Biosorption experiment set-up

Phytotoxicity test of leachate

White mustard (*Sinapis alba* L.) was used as a test organism to assess the toxicity of the leachate samples before and after the *P. ostreatus* biosorption experiment. Each leachate sample was diluted to give final leachate concentrations of 25%, 50%, 75% and 90%. Each concentration of the dilution series was tested with two replicate samples. The test organisms were exposed to the leachate solutions for a total of 72 h.

The seeds of *Sinapis alba* L. were germinated in Petri dishes (Figure 2) with a 14 cm diameter on filter paper on the bottom. The hydroponic solution

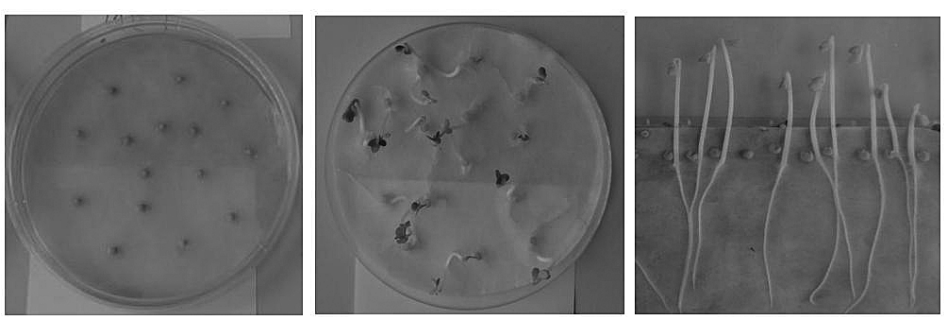


Fig. 2. Process of *Sinapis alba* L. seed germination and growth

(distilled water with the following chemical ingredients (mg l^{-1}): $Ca(NO_3)_2$ 0.8, KH_2PO_4 0.2, KNO_3 0.2, $MgSO_4 \cdot 7H_2O$ 0.2, KCl 0.2, $FeSO_4$ 0.01, pH = 5.2) with the tested liquid was added into each dish, and 15 healthy looking seeds of similar size were evenly spread onto the surface of the filter paper. The Petri dishes were covered by a glass cap to prevent loss due to evaporation and were located in the dark thermostat (t = 24°C, air humidity 80%). After 72 h, the root length was measured.

Statistical analysis

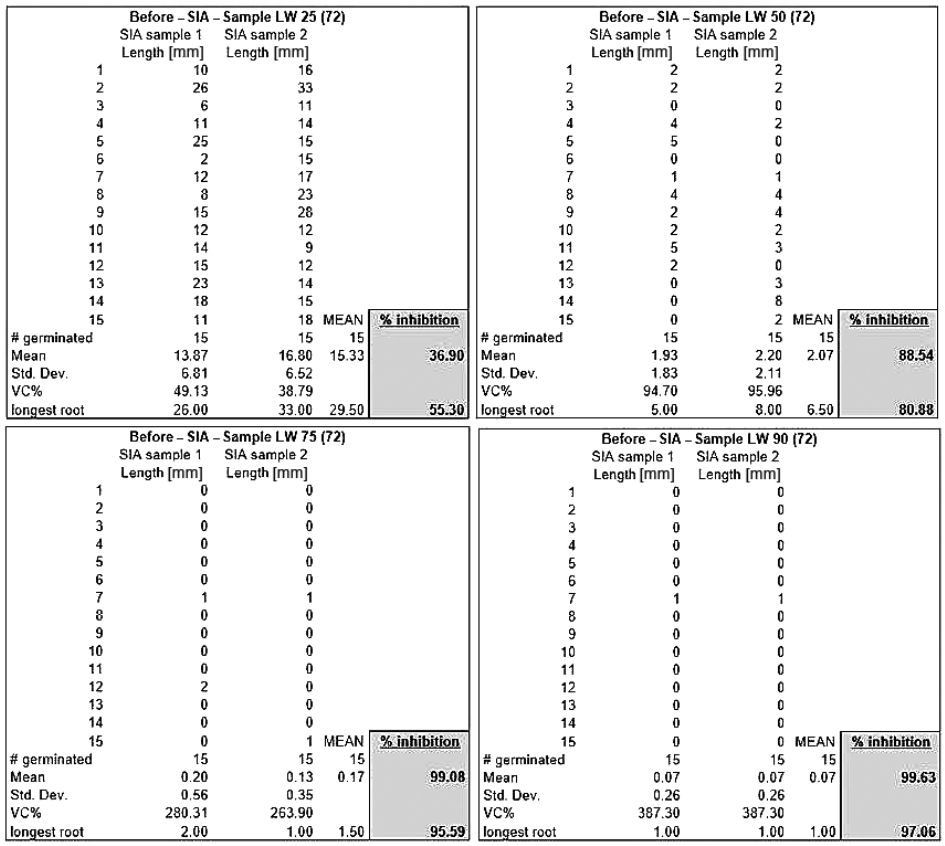
Toxicity data were expressed as the percentage of toxic effect of leachate compared to the control. Mean values and standard deviations (±SD) were taken from each two data set. Statistical analysis was performed using the software Excel.

Results and Discussion

The present study describes the efficiency of *P. ostreatus* as a biosorbent for the removal of toxicity from aqueous solutions – landfill leachate (LW). Figure 3 and Figure 4 present the results of root length [mm] and inhibition [%] for samples before LW 25, LW 50, LW 75 and LW 90 and after LW 25, LW 50, LW 75 and LW 90 *P. ostreatus* biosorption tests using the *Sinapis alba* L. growth inhibition test.

Figure 5 presents the effect of the landfill leachate samples before and after *P. ostreatus* biosorption tests (concentration 25% (LW 25), 50% (LW 50), 75% (LW 75) and 90% (LW 90)) on the inhibition of seed germination and root growth as related to the test plants *Sinapis alba* L. (SIA). The growth inhibition (%) of the *Sinapis alba* L. for landfill leachate samples Before – LW 25, LW 50, LW 75 and LW 90 were in the range of 36.90–99.63%. These samples were strongly toxic. It can be concluded that the higher the concentration of landfill leachate, the greater the inhibition.

The growth inhibition (%) of the *Sinapis alba* L. for landfill leachate samples after biosorption tests, samples After – LW 25, LW 50, LW 75 and LW 90 were in the range of 31.55–96.16%. These samples were strongly toxic, but the toxicity compared to the samples before – LW 25, LW 50, LW 75 and LW 90 decreased for all samples. After the *P. ostreatus* biosorption tests the toxicity of landfill leachate decreased, for sample LW 25 by 15%, for sample LW 50 by 1%, for sample LW 75 by 4.3% and form sample LW 90 by 3.48%. Samples After LW 25, LW 50, LW 75 and LW 90 showed an average of 5.9% lower toxicity than samples Before LW 25, LW 50, LW 75 and LW 90.



Before – SIA – Sample LW 25 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	10	16		
2	26	33		
3	6	11		
4	11	14		
5	25	15		
6	2	15		
7	12	17		
8	8	23		
9	15	28		
10	12	12		
11	14	9		
12	15	12		
13	23	14		
14	18	15		
15	11	18	MEAN	% inhibition
# germinated	15	15	15	
Mean	13.87	16.80	15.33	36.90
Std. Dev.	6.81	6.52		
VC%	49.13	38.79		
longest root	26.00	33.00	29.50	55.30

Before – SIA – Sample LW 50 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	2	2		
2	2	2		
3	0	0		
4	4	2		
5	5	0		
6	0	0		
7	1	1		
8	4	4		
9	2	4		
10	2	2		
11	5	3		
12	2	0		
13	0	3		
14	0	8		
15	0	2	MEAN	% inhibition
# germinated	15	15	15	
Mean	1.93	2.20	2.07	88.54
Std. Dev.	1.83	2.11		
VC%	94.70	95.96		
longest root	5.00	8.00	6.50	80.88

Before – SIA – Sample LW 75 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	0	0		
2	0	0		
3	0	0		
4	0	0		
5	0	0		
6	0	0		
7	1	1		
8	0	0		
9	0	0		
10	0	0		
11	0	0		
12	2	0		
13	0	0		
14	0	0		
15	0	1	MEAN	% inhibition
# germinated	15	15	15	
Mean	0.20	0.13	0.17	99.08
Std. Dev.	0.56	0.35		
VC%	280.31	263.90		
longest root	2.00	1.00	1.50	95.59

Before – SIA – Sample LW 90 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	0	0		
2	0	0		
3	0	0		
4	0	0		
5	0	0		
6	0	0		
7	1	1		
8	0	0		
9	0	0		
10	0	0		
11	0	0		
12	0	0		
13	0	0		
14	0	0		
15	0	0	MEAN	% inhibition
# germinated	15	15	15	
Mean	0.07	0.07	0.07	99.63
Std. Dev.	0.26	0.26		
VC%	387.30	387.30		
longest root	1.00	1.00	1.00	97.06

Fig. 3. Root length (mm) and inhibition results (%) of samples before LW 25, LW 50, LW 75 and LW 90

Fungi can be used to treat a variety of wastewaters, ranging from municipal wastewater, industrial wastewater and landfill leachate. In terms of landfill leachate treatment, fungi showed a better removal efficiency of recalcitrant compounds than the conventional leachate treatment process (REN and YUAN 2015).

The biosorptive capacity of dead fungal cells has been studied extensively in comparison to living cells. The biosorptive capacity of dead cells may be greater, equivalent to or less than that of living cells. The use of dead biomass in industrial applications offers certain advantages over living cells. Systems using living cells are likely to be more sensitive to metal-ion concentration (toxicity effects) (KAPOOR and VIRARAGHAVAN 1995).

The effect of biosorbent was also studied by JAVAID et al. (2011). The study concluded that the fungal species *P. ostreatus* was an efficient biosorbent

After – SIA – Sample LW 25 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	10	16		
2	26	33		
3	16	11		
4	11	14		
5	25	15		
6	2	15		
7	12	17		
8	14	23		
9	15	28		
10	12	12		
11	14	22		
12	15	12		
13	23	14		
14	18	15		
15	21	18	MEAN	% inhibition
# germinated	15	15	15	
Mean	15.60	17.67	16.63	31.55
Std. Dev.	6.30	6.26		
VC%	40.38	35.46		
longest root	26.00	33.00	29.50	55.30

After – SIA – Sample LW 25 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	0	5		
2	0	6		
3	6	4		
4	7	3		
5	4	7		
6	0	6		
7	6	5		
8	5	0		
9	0	0		
10	4	5		
11	6	0		
12	0	0		
13	5	0		
14	0	0		
15	6	0	MEAN	% inhibition
# germinated	15	15	15	
Mean	3.27	2.73	3.00	87.65
Std. Dev.	2.87	2.79		
VC%	87.71	102.05		
longest root	7.00	7.00	7.00	89.39

After – SIA – Sample LW 25 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	0	5		
2	0	0		
3	5	4		
4	0	3		
5	4	0		
6	0	0		
7	2	5		
8	0	0		
9	0	0		
10	0	5		
11	0	0		
12	0	0		
13	5	0		
14	0	0		
15	0	0	MEAN	% inhibition
# germinated	15	15	15	
Mean	1.07	1.47	1.27	94.79
Std. Dev.	1.94	2.20		
VC%	182.29	149.97		
longest root	5.00	5.00	5.00	92.42

After – SIA – Sample LW 25 (72)

	SIA sample 1 Length [mm]	SIA sample 2 Length [mm]		
1	0	0		
2	0	0		
3	3	0		
4	0	3		
5	2	0		
6	0	0		
7	2	5		
8	0	0		
9	0	0		
10	2	4		
11	2	0		
12	0	0		
13	5	0		
14	0	0		
15	0	0	MEAN	% inhibition
# germinated	15	15	15	
Mean	1.07	0.80	0.93	96.16
Std. Dev.	1.53	1.70		
VC%	143.79	212.34		
longest root	5.00	5.00	5.00	92.42

Fig. 4. Root length [mm] and inhibition results [%] of samples after LW 25, LW 50, LW 75 and LW 90

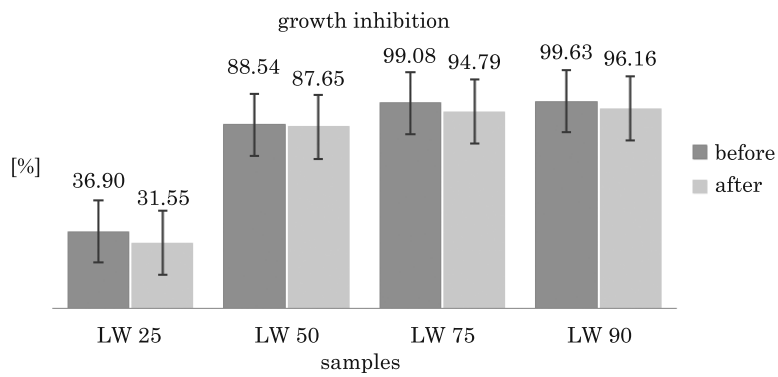


Fig. 5. Growth inhibition in samples LW 25, LW 50, LW 75 and LW 90 (before and after)

because of fast metal removal rate, remarkable biosorption capacity and high regeneration ability. Treatment of real effluents corroborated that *P. ostreatus* has a good potential to remove toxic heavy metal ions from industrial effluents in real applications (JAVAID et al. 2011). In another study it was shown that the fruiting bodies of *P. ostreatus* immobilized in calcium alginate were effective in removing lead from solution efficiently (XIANGLIANG et al. 2005).

Conclusion

The results presented in this paper demonstrate the usability of a biosorbent, *P. ostreatus*, for the removal of landfill leachate toxicity. This study is part of the larger research of long-term landfill monitoring program. The growth inhibition [%] of *Sinapis alba* L. for landfill leachate samples after biosorption tests were in the range of 31.55–96.16%. These samples were strongly toxic, but the toxicity compared to samples before biosorption tests decreased for all samples. Biosorbent, *P. ostreatus* may find potential application in landfill leachate treatment.

Translated by HERMES VILLAFAÑA

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SELECTED THERMAL AND BIOTHERMAL ASPECTS OF CITIES IN POLAND

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K e y w o r d s: agglomeration, sensation, load, stress.

A b s t r a c t

Living conditions, place of residence and surrounding natural as well as atmospheric environment determine human health status and life expectancy. This article presents the specificity of biothermal conditions as illustrated by the selected cities in Poland, with a particular consideration of unfavourable thermal conditions due to excessively high or low air temperature. The present article is a review. In accordance with the tendencies of climate change, it was found that in the cities in Poland, conditions hazardous to health and life due to excessively high temperature and heat stress as well as very strong heat stress, occur with increasing frequency, particularly in the city centre. Additionally, thermal discomfort which is markedly present in the city centre is aggravated and moved to night-time due to urban heat island. According to numerous studies, the incidence of such situations is less frequent in the coastal zone and in the suburban zones.

WYBRANE ASPEKTY TERMICZNE I BIOTERMICZNE MIAST W POLSCE

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A b s t r a k t

Warunki życia, miejsce zamieszkania, otaczające środowisko przyrodnicze i atmosferyczne determinują stan zdrowia i długość życia człowieka. W opracowaniu przedstawiano specyfikę warunków biotermicznych na przykładzie wybranych miast Polski, ze szczególnym uwzględnieniem

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występowania niekorzystnych warunków termicznych związanych ze zbyt wysoką lub zbyt niską temperaturą powietrza. Praca ma charakter przeglądowy.

Wykazano, że w polskich miastach, zgodnie z tendencjami zmian klimatu, mamy do czynienia z większą częstością występowania niebezpiecznych dla zdrowia i życia, szczególnie w centrum, sytuacji związanych ze zbyt wysoką temperaturą powietrza i występowaniem zjawiska stresu gorąca i bardzo gorąca. W centrum miast uwidacznia się także niekorzystny dyskomfort cieplny potęgowany i przesuwany na godziny nocne z powodu występowania miejskiej wyspy ciepła. Sytuacje takie, jak wskazują wyniki wielu prac, są natomiast rzadsze na obrzeżach czy w strefie podmiejskiej.

Introduction

The health of individuals as well as communities is highly dependent on environmental factors, including atmospheric factors (KOZŁOWSKA-SZCZĘSNA et al. 2004, KOZŁOWSKA-SZCZĘSNA and BŁAŻEJCZYK 2010). According to the WHO report (2016), in 2010 people living in cities constituted 70% of the total population. The number is estimated to reach 80% by 2045. Exposure to various factors affecting health and life of the population is even greater in anthropogenically changed environment. According to the World Health Organization reports (WHO 2006 and 2016), over the last 10 years, the incidence of illnesses attributed to changes of environment due to anthropogenic pressure remained at a comparable level and constituted approximately 23–24% of the global burden of disease. According to BŁAŻEJCZYK and MCGREGOR (2007), 15–30% of all deaths in Europe per year can be coupled with specific values of biothermal indices recorded 1–3 days before. Markedly increased health and life risk, particularly among the highly vulnerable group, is observed during the heatwave. Therefore, it is alarming to find an increase in the frequency of extremely high air temperature values and its duration as well as intensity of heatwaves observed over the last decades globally and hemispherically (PERKINS et al. 2012), as well as in individual regions of the world, including Europe (DELLA-MARTA et al. 2007, PETERSON et al. 2013) and Poland (WIBIG et al. 2009, BIELEC-BĄKOWSKA and PIOTROWICZ 2013). This negative trend is projected to continue in the future (MEEHL and TEBALDI 2004, KYSELÝ 2010, IPCC 2014). HÜBLER et al. (2008) state that in the period 2071–2100, approximately 80,000 people from the member states of the European Union will have died due to hyperthermia. In turn, according to the projection by BŁAŻEJCZYK et al. (2016) taking into account the assumed scenarios for greenhouse gas emission, till the year 2100, the number of deaths due to heat stress is believed to increase (by 137–277%) and the risk of death due to hypothermia as well as the number of deaths due to health effects of cold stress is believed to decrease (by 23–50% and 64–74%, respectively). That is why measures are being taken to minimise the negative manifestations of the forecasted change in climate. The example of such actions is *Development and application of mitigation and*

adaptation strategies and measures for counteracting the global Urban Heat Island phenomenon focused on developing a strategy to reduce and prevent risks and manage urban heat islands in the central Europe developed in 8 of the most relevant metropolitan areas and MEGAs (Mega Urban Regions): Bologna – Modena (IT), Venice – Padua (IT), Wien (AT), Stuttgart (DE), Lodz and Warsaw (PL), Ljubljana (SI), Budapest (HU), Prague (CZ). UHI Project wants to boost transnational discussion among policy makers, local administrators and professionals that will bring developing policies and actions for preventing, adapting and mitigating the natural and man-made risks arising from the UHI phenomenon (*UHI* 2017). In turn, the European Commission has demonstrated in the *EU Strategy on adaptation to climate change* of April 2013 that in order to prepare the member states to foreseen climate change, it is necessary to take actions on all levels – national, regional and local. The local actions to be taken, concerning the city areas, include development of Urban Adaptation Plans to climate change, which comprise both hazard analysis as well as suggestions on adaptive measures to be taken. The European Environmental Agency, through its platform Climate-Adapt, provides a series of interactive maps presenting threats to cities (heatwaves, water shortages, drought, floods and wildfire) due to climate change, and shows the capability of the cities to react to such changes.

The present paper concerns spatial variability of the so-called thermal specific days and night, and heat load determined with UTCI index (Universal Thermal Climate Index) in selected cities in Poland.

Thermal specific days

One of the principle and widely adopted characteristics used to describe thermal conditions in a given place or area, apart from mean multi-annual air temperature value, is the number of days with maximum or minimum temperature within the set range of values. Such days are usually referred to as thermal specific days and are identified by air temperature exceeding the set range of values (e.g. very hot day t_{max} from > 30.0°C to 35.0°C or cold day t_{max} < 0.0°C to -10.0°C).

Occurrence of days with particularly low or high air temperature contributes significantly to health status of population (results in cold, frostbite, hyperthermia, heat stroke). From the perspective of urban areas, thermal specific days connected with high temperature values are of greater importance. As was demonstrated by MATUSZKO and PIOTROWICZ (2015), since 1910 in Kraków the number of cold days has decreased, and the frequency of strenuous very hot days has increased. Similarly, a negative trend for not only cold but

also very cold days as well a positive trend for hot and very hot days was found in Warsaw by MAJEWSKI et al. (2012) and KOSSOWSKA-CEZAK (2014). A positive trend in the number of hot days and sporadic cases of very hot days in the cities of the coastal zone was discussed by KOŹMIŃSKI and MICHALSKA (2011). In turn, PODSTAWCZYŃSKA (2010) points out that the positive trends in extremely hot days have been observed particularly since 1980.

Figure 1 shows the number of hot, very hot and extremely hot days in 16 cities in Poland in August 2015. The increase in very hot days and a decrease in the number of hot days marked from the north-west towards the south is noteworthy.

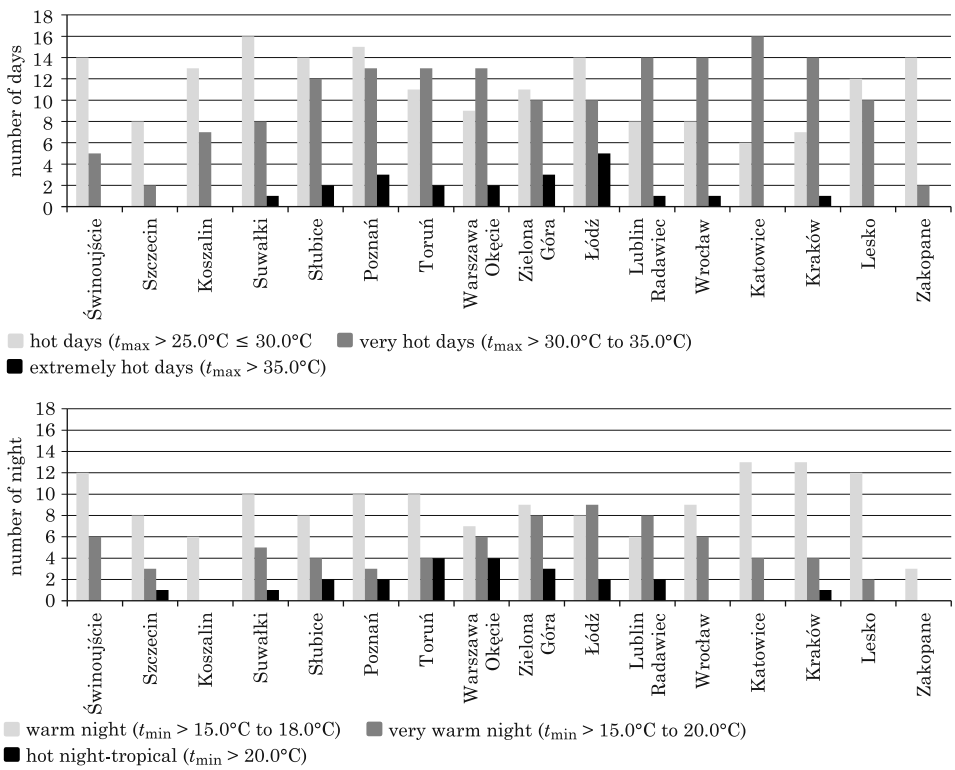

Fig. 1. The number of thermal specific days and nights in selected cities in Poland – August, 2015

A series of thermal specific days lasting several days is a significant indicator of thermal regime. Urban areas are particularly subject to prolonged and ever more intense heatwaves, which (taking into consideration the diversity of identification methods) should be understood as periods of at least

several days in which air temperature is markedly higher than that characteristic for a given area. According to IPCC projections (2014), heatwaves are believed to last longer, show greater severity and occur more frequently. Despite various methods of identifying the heatwaves, the exhibited tendencies are consistent and show that nowadays the incidence of heatwave has even doubled (KRZYŻEWSKA 2015, WIBIG et al. 2009). In the climatic conditions of Poland, very hot days are recorded throughout the country except for the mountain areas. For example, in Warsaw in the period 1947–2010 there were 35 heatwaves, the longest lasting 10 and 9 days (KOSSOWSKA-CEZAK and SKRZYPCZUK 2011). In turn, in Poznań in the period 1971–2010 there were 34 heatwaves and the longest heatwave lasted 18 days (TOMCZYK 2015), and in Lublin in the period 1951–2010 there were 30 heatwaves with the longest lasting 10 days (KRZYŻEWSKA 2015). In the cities located in the coastal zone, heatwaves are recorded much less frequently, for example in the period 1986–2009 KOŹMIŃSKI and MICHALSKA (2011) identified only from 5 heatwaves in Ustka to 9 in Kołobrzeg, and their duration was maximum 3 days. During the heatwave of 1994 in Warsaw, total mortality increased by 33% in comparison to respective period in 1995 (KUCHCIK 2001). The heatwave of 1994 was marked by the longest duration over most Central Europe and, among others, in Ukraine (TOMCZYK 2015). In turn, following the extremely hot summer of 2003 when the number of deaths was 35,000 including 14,000 in France (TOMCZYK 2015), there has been a revived interest among researchers in very hot weather both as an extreme phenomenon as well as in relation to human health and life. According to TWARDOSZ (2009), in Paris, in only one day (13th August 2003) the number of reported deaths exceeded the daily average by 600%. It is worth noting, that the heatwave of 2003 was accompanied by drought which had serious impact on agricultural economy (GARCÍA-HERRERA et al. 2010), and in 2010 it coincided with devastating wildfire and resulting increase in air pollution (BARRIOPEDRO et al. 2011).

The examples presented above, that is more frequent occurrence of very hot days and heatwaves as well as a decrease in the number of cold and very cold days, is generally considered as a manifestation of ongoing global warming which has also been observed in Poland by numerous authors (e.g. CEBULAK and LIMANÓWKA 2007, KEJNA et al. 2009, BIELEC-BĄKOWSKA and PIOTROWICZ 2013, ŁUPIKASZA et al. 2014, TOMCZYK and BEDNORZ 2014).

In urban areas, particularly significant is the phenomenon of Urban Heat Island (UHI) which is especially negative in the summer period. In particularly hot and very hot periods, UHI hinders heat transfer and negatively affects regeneration of the organism in the evening and at night. Depending on intensity and adopted criteria, the nights with high minimum temperature are called warm, very warm or even tropical nights. The increasing frequency

of such nights in the cities in Poland and the resulting burden was discussed by: KOSSOWSKA-CEZAK and SKRZYPCZUK (2011), MATUSZKO and PIOTROWICZ (2012), BIELEC-BĄKOWSKA and PIOTROWICZ (2013), BŁAŻEJCZYK et al. (2014), TOMCZYK (2015), WIĘCŁAW (2015), observe that due to its specific nature, UHI can even increase the effect of heatwave through limited or absent temperature reduction in the evening and night-time. This is because the lack of regeneration in the night-time (relief from heat in cooler environment) causes the effect of overlapping heat stress for an organism in consecutive very hot days. The incidence of minimum air temperature being higher than the assumed value threshold is termed as a thermal specific night, similarly to the thermal specific days mentioned above. For example, a night is termed very warm when minimum temperature is from 18.1 to 20.0°C, and tropical (also called very hot) when the temperature is over 20.0°C. In different cities in Poland, especially over the past dozen or so years, the frequency of unfavourable thermal conditions at night-time is increasing (MATUSZKO and PIOTROWICZ 2012, KOSSOWSKA-CEZAK 2014, WIĘCŁAW 2015). Apart from conditions of air circulation, particularly advection of tropical and polar-continental air masses, the authors attribute the increasing frequency to anthropogenic factors. It is worth noting that, similarly as in the case of hot days and heatwaves, exposure to heat, measured as the number of hot days and tropical nights, is projected to increase across Europe. Cities in southern Europe are most exposed, but these hot days and tropical nights are also projected to increase in western, central and eastern Europe, where people and towns are less accustomed to heat (*European Climate...* 2017). Figure 1 presents the exemplary number of thermal specific nights in August in selected cities of Poland.

Biothermal conditions

Urban areas are characterised by specific modifications of given meteorological elements (FORTUNIAK 2003, SZYMANOWSKI 2004, BŁAŻEJCZYK et al. 2014, NIDZGORSKA-LENCEWICZ and MĄKOSZA 2016), thus the bioclimatic conditions change (BŁAŻEJCZYK and KUNERT 2010, CZARNECKA et al. 2011) and differ from the conditions found in the suburban areas. The specificity of bioclimate of the cities is most evident in sensations and heat load, as well as in aerosanitary conditions. Projected scenarios of climate change show that the increase in temperature, both on a global as well as local scale, is reflected in the increase in heat load particularly in the urban areas (IPCC 2014). Thermal sensation and heat load result from temperature but also are shaped by incidence of long-wave and short-wave radiation, humidity and wind speed. This multifactorial influence and effect on human organism is presented with the use

of various biothermal indices and man – environment heat exchange models. Nowadays, Polish researchers analysing bioclimate in cities most often employ indices based on human heat balance and, recently predominant, Universal Thermal Climate Index (UTCI). According to the authors of UTCI index (BŁAŻEJCZYK et al. 2010, BRÖDE et al. 2012), it is defined as the equivalent value of air temperature at which, in reference conditions, the basic physiological parameters of an organism take the same values as in real conditions. The index has a thermal dimesion (°C) and allows determination of heat load in various thermal conditions of the environment. Individual range values of UTCI determine particular categories of heat stress based on objective changes in physiological parameters of an organism due to conditions of the environment, and constitute a measure of heat load.

The studies on the analysis of UTCI index regarding biothermal conditions in Polish cities present the values of the index as well as frequency of individual stress categories across various temporal and spatial terms. It was found that in most cities in Poland, per year and regardless of region and adopted time-frame of the studies, the predominant category of thermal stress is lack of heat load which amounts to 40%, with approximately 60% in the warm half-year and merely 1–5% in the cold half-year (LINDNER 2011, MĄKOSZA 2013, DOBEK and KRZYŻEWSKA 2015, MĄKOSZA et al. 2015, NIDZGORSKA-LENCEWICZ 2015, ROZBICKA and ROZBICKI 2017). The same studies show that moderate or low cold stress is recorded with comparable frequency (20–40%). The extreme UTCI index categories, which have the greatest effect on human organism, show greater difference in terms of frequency. For example, in the coastal zone cities, days with strong and very strong heat stress are recorded with 0.3–1.5% frequency in a year (NIDZGORSKA-LENCEWICZ 2015, PÓŁROLNICZAK et al. 2016), whereas in the west of Poland (Szczecin, Gorzów Wlkp., Słubice, Zielona Góra) such days are recorded more frequently – 0.6–2.3% (MĄKOSZA 2013, NIDZGORSKA-LENCEWICZ and MĄKOSZA 2013), in Warsaw the frequency amounts to approx. 1.6% (ROZBICKA and ROZBICKI 2017), and in Lublin – 1% (DOBEK and KRZYŻEWSKA 2015). Illustrative frequency of various UTCI stress categories observed in 2006 in Gdańsk, Warsaw and Katowice is given in Figure 2 which clearly demonstrates the aforementioned relationship.

The studies on spatial variability of biothermal conditions such as BŁAŻEJCZYK et al. (2014) for Warsaw, NIDZGORSKA-LENCEWICZ and MĄKOSZA (2013) for Szczecin, and ARAŹNY et al. (2016) for Toruń, the city centre is marked by slightly more frequent occurrence of heat load due to heat stress, and cold stress is predominant in the suburban areas. However, the load varies depending on the characteristics of a city under analysis. In Szczecin, in was found that various categories of heat stress occur with 5% frequency in the city

centre, to 1% frequency in the southern suburban area. By comparison, heat stress in Warsaw is recorded on 19% of days in the city centre, and only 4% of days in the suburban areas. Cold stress is much more frequent in the climatic conditions of Poland. In Szczecin, cold stress was identified on 64% of days in the city centre, and 73% in the suburban area whereas in Warsaw city centre cold stress occurred on 39% of days in a year and 60% in the suburban area. In both cities, cold stress was usually mild or moderate. In the city centre, very strong cold stress was not recorded in Warsaw and in Szczecin it constituted but a fraction of incidence, yet in suburban area the frequency was 2% of days in both cities. Exemplary course of UTCI values presenting differences between the city centre and the suburban area in January and July 2006 in Szczecin is shown in Figure 3.

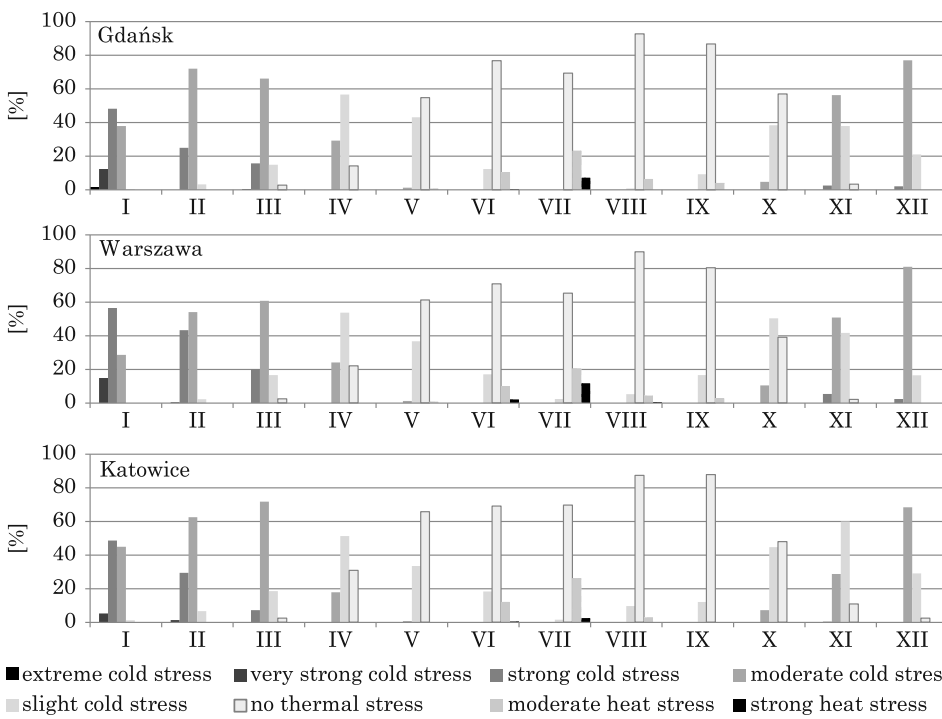

Fig. 2. Frequency of thermal stress class according to UTCI index in Gdańsk, Warszawa and Katowice – year 2006

IDZIKOWSKA (2011) attempted to show the relationship between UTCI values and mortality in Paris, Rome, Warsaw and Budapest indicating that the strongest relationship was found in the summer months (July and August).

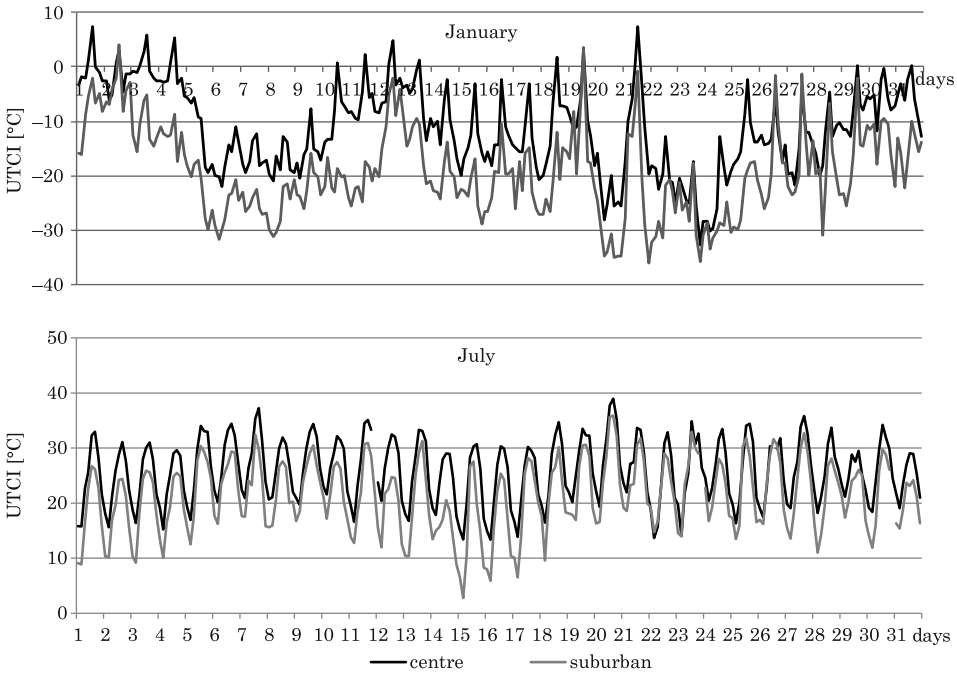

Fig. 3. The hourly UTCI index values in January and July 2006 year in Szczecin city

The author has also demonstrated a 2–3 days delay in the increase in the number of deaths as compared to UTCI values – the so-called delay effect, which is in line with the findings by BŁAŻEJCZYK and MCGREGOR (2007).

Conclusions

In urban areas, modifications of atmospheric conditions found in the city centre result in incidence of variable conditions of thermal sensation, particularly bioclimatic conditions. An increase in the number of thermally unfavourable days and night and increasing frequency of heat stress were found. As the authors of the aforementioned papers demonstrate, a particular role in the context of biothermal conditions and human health and well-being is played by the phenomenon of urban heat island. Therefore, of particular importance are the adaptation actions taken by the cities in relation to assumed or expected change in bioclimatic conditions. Such actions, in turn, require a comprehensive and integrated cooperation in terms of social, economic, environmental and spatial context included in planning documents (KLIMADA 2016). In the context of frequent incidence of unfavourable biothermal conditions due to

very strong heat stress (very hot day), of particular importance are biologically active areas. According to the New Charter of Athens (2003), in the 21st century cities, care will be taken to provide the residents with close proximity of residence area and place of work, cultural facilities, natural heritage areas such as parks, squares, open green space etc. Given the various climate change scenarios and increase in maximum temperature, the Adaptation Strategies for Climate Change in the Urban Environment (ASCCUE) present, among others, the possibility of decreasing temperature by 0.8–1.2°C on highly urbanised area by means of increasing the biologically active area by 10%. At the same time, a decrease in biologically active area by 10% would result in the opposite effect, i.e. an increase in temperature by as much as 7.0–8.2°C (BŁAŻEJCZYK et al. 2014).

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