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EVALUATION OF THE IMPACT OF PRE-SOWING MICROWAVE STIMULATION OF BEAN SEEDS ON THE GERMINATION PROCESS

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ABSTRACT

The objective of the paper was to evaluate the impact of microwave stimulation of bean seeds on their germination process. Laboratory tests were carried out in 2012-2014 with the use of certified bean seeds (*Phaseolus vulgaris* L.) of Igołomska cultivar. Shortly before sowing, bean seeds were radiated with microwaves for 10, 30 and 60 seconds. Microwaves came from a magnetron which operated with 100 W power and produced waves of 2.45 GHz frequency. The germination process was described and parameters, which define the sowing value of bean seeds, were determined. Germination ability, relative germination ability (Maguier's index) and average germination time (Pieper's index). Fresh and then dry mass of plant mass were determined. The obtained results allow the statement that microwaves modify the germination process of bean seeds.

The list of abbreviations and symbols:

- M_f – mass of a single bean seed, (g)
- φ_f – coefficient of shape of a single bean seed, (-)
- P_b – water volume of tissue paper, (-)
- S_n – degree of water saturation of a germination bed, ($S_n = 0.5$ to 1.0) (-)
- M_b – mass of tissue paper in the germination box, (g)
- \dot{S}_{mf} – fresh mass of a bean plant, (g)
- S_{mf} – dry mass of a bean plant, (g)
- U_{mf} – mass loss of a bean plant, (g)
- W_z – coefficient of variation, (%)
- S_d – standard deviation,
- min., max., average – respectively: maximum value, minimum value and average
- l – number of germinated seeds in subsequent days of observation,
- d – number of a day from seeds dissemination,
- η_p^2 – (partial eta square) measure of size of experimental effects.

Introduction

Health of seeds is one of fundamental properties which prove its quality and usefulness in agriculture. Next to treating seeds with crop protection chemicals or immersion in chemical solutions also physical methods of their disinfection are applied (removing part of a seed cover habited by pathogens, thermal activity, the use of electromagnetic or mechanical waves of varied frequency) (Ispir and Togrul, 2009; Kaniewska et al., 2012). On account of legal regulations of the European Parliament concerning introduction (restrictions) of crop protection chemicals to turnover (Regulation EP 1107, 2009) it is recommended to search for seeds disinfection methods which are new and at the same time such that do not deteriorate the germination vigour. Bean seeds, on account of the content of polyphenols are antioxidant (a functional property) (Drużyńska and Klepacka, 2004). At the same time Chmielowiec and Borowy (2004) emphasise that Poland is the third producer of dry bean seeds in Europe and in the recent years in our country, bean cultivation for dry seeds was approximately 20 thousand hectares (Łabuda, 2010). According to Villavicencio et al. (2000) bean seeds are one of the main sources of protein in a diet in developing countries. Cultivation of beans poses also some difficulties and one of them is obtaining fast and high crops, since in the period from sowing to germination, seeds are exposed to decay caused by the pathogens activity (Szafirowska and Kołosowski, 2008). Both seeds as well as young seedlings may be the place where pests live (e.g. *Hylemyia florilega* Zett., *Delia platura* Meig). Participation of seeds in the mass of a whole plant along with its maturation of stems, decreases. Approximately 24-55% of the total dry mass of bean plants is stored in bean pods (Halepyati and Ali, 1993). Undoubtedly, physical methods of its protection are an alternative for chemicalization of seeds. Refining seeds should not only increase its vigour or limit variability of physical and chemical properties but also favourably affect the growth and development of a plant in further stages of its ontogenetic development (Wójcik et al., 2004). Radiation of seeds with microwaves is one of physical methods of stimulation of seeds (Olchowik et al., 2002). In the world scientific literature results of research concerning a positive influence of microwaves on lentil seeds (Aladjadjiyan, 2010), rapeseed (Opricá, 2008) and soya, wheat, barley and oats (Reddy et al., 1995, 1998; Yoshida et al., 2000; Ponomarev et al. 1996) were discussed. The research carried out by Tylkowska et al. (2010) shows that microwave radiation (with the frequency of 650 W and 2450 MHz) used with reference to bean seeds (*Phaseolus vulgaris*) of Laurina cultivar limited occurrence of *Penicillium* spp. fungi, both on the surface as well as outside seeds. Similarly Friesen et al. (2014) stated that radiation of bean seeds (*Phaseolus vulgaris*) of Navigator and Ole cultivars with microwaves (1100 W and 2450 MHz) reduces infection of plants with *Colletotrichum lindemuthianum* (which causes bean anthracnose). In the research, which was presented above, it was not found out whether microwaves modify the germination process of bean seeds. The author's research (Jakubowski, 2010a) concerning the effect of microwaves (100 W and 2450 MHz) on potato plants indicate that radiation of the bulb affects the process of its germination at the simultaneous decrease of the degree of infection with canker (Jakubowski, 2010b). Thus, there is a probability that microwave radiation of bean seeds with 2450 MHz frequency generated by a magnetron operating at 100 W power, will modify the process of plant germination. The objective of the paper was to evaluate the impact of microwave stimulation of bean seeds on the germination process.

The scope of paper and the methodology of research

Laboratory tests were carried out in 2012-2014 with the use of certified bean seeds (*Phaseolus vulgaris* L.) of Igołomska cultivar. It is a prolific very early variety with white averaged size seeds. Sowing is performed the most often in May and June (in a greenhouse cultivation it may be as early as in April). Material for tests was obtained from Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN Spółka z o.o.

$$\varphi = \sqrt[3]{\frac{a \cdot b \cdot c}{c^3}} \quad (1)$$

In order to present the uniformity of the research material, mass (M_f) and the coefficient of shape (φ_f) of a single bean seed (acc. to formula 1) was determined. The mass was determined with the use of a laboratory balance (Radwag XA 110/X, $d=0.01$ g) and dimensions of seeds with the use of a caliper (DIN 862 +/- 0.03 mm) coupled with a data registration unit (measurement). Each combination of the experiment included 3 replications. The experiment included investigation of differences between replications within each combination and then differences between combinations. It was assumed after Anders (2003) that replication is sufficient when it meets the following conditions: values of the coefficient of variation W_z for variables M_f i φ_f will not exceed respectively 10 and 15% (Table 1) and the distribution of the investigated separation property showed the properties of regular distribution (or logarithmic – regular). From the point of view of methodology, this manner of selection of a sample was indicated because Lima et al. (2005) proved relations between the size of a bean seed and its growth, development and cropping. Adjusting empirical distribution of the sample to the regular theoretical distribution (logarithms-regular) was carried out with Kolmogorov-Smirnov test. Shortly before sowing, bean seeds, placed on the petri dish were radiated with microwaves for 10, 30 and 60 seconds. Microwaves came from a magnetron which operated with 100 W power and produced waves of 2.45 GHz frequency. In order to determine energy absorbed by a single bean seed during microwave radiation a unit dose of microwave radiation was calculated (the amount of the total dose of microwave radiation and the mass of bean seeds. The total dose of microwave radiation was defined as the power product of a device which generates microwaves and the exposition time. After irradiation, seeds were placed in plastic containers which serve as a germination apparatus. On the bottom of each germination apparatus a layer of lignin was placed (M_b), which was moistened with water (M_w). According to the relation (2) in the initial phase of experiment, the degree of water saturation of lignin (S_n) equal to 0.8 was obtained (Domoradzki, 1999).

$$M_w = P_b \cdot M_b \cdot S_n \quad (2)$$

In the so prepared germination apparatus, seeds were placed, which were later left without light for 48 hours in temperature of 22°C. For the following 8 days, seeds were germinated with daylight in temperature of 20-22°C. Each day (according to PN-R-65950: 1994 and ISTA standards the number of seeds with a growing germinal root, which pierced all coats, actively elongates and geotropically curves was determined – a visible primary root or hypocotyl) and water in germination apparatuses was regularly filled up. Total period of time of the experiment (initial sprouting germination) was selected to include three basic stages of germination (imbibition stage (2 days) and catabolic and anabolic stage (remain-

ing 8 days). The process of germination was presented graphically and the following parameters were determined (Gładyszewska, 2004; Binek and Moś, 1984) which define the sowing value of bean seeds:

- germination ability of the investigated bean seeds was defined as a percentage of normally germinated seeds within the standard time,
- Maguier's index (W_{Magu}) which determines a relative germination speed (3),

$$W_{Magu} = \frac{l_1}{d_1} + \frac{l_2}{d_2} + \dots + \frac{l_n}{d_n} \quad (3)$$

- Pieper's index (W_{Piep}) which determines average time of germination of a single bean seed (4).

$$W_{Piep} = \frac{(l_1 \cdot d_1 + l_2 \cdot d_2 + \dots + l_n \cdot d_n)}{(l_1 + l_2 + \dots + l_n)} \quad (4)$$

On the tenth day, germinated seeds were taken out of germination apparatuses along with roots and the fresh and then dry mass of plants was determined. In order to determine fresh mass (\dot{S}_{mf}) strained plants were weighted one by one on a laboratory balance (Radwag XA 110/X, $d=0.01$ g). In order to determine dry mass of plants (S_{mf}) samples were dried in temperature of 105°C in a laboratory drier for 5 hours and then their weight was determined (analogically to the description above). The loss of plants mass (U_{mf}) resulting from drying was determined as a difference $\sum \dot{S}_{mf}$ and S_{mf} .

Table 1
Values of basic statistics for the used experimental material (example for 2012)

Radiation time	Replication no.	Mass (g)					Coefficient of shape				
		min	max	aver.	Sd	Wz	min	max	aver.	Sd	Wz
0 s (control)	1	0.37	0.45	0.41	0.04	10.0	0.630	0.639	0.634	0.091	14.3
	2	0.38	0.44	0.41	0.03	7.5	0.631	0.647	0.639	0.072	11.3
	3	0.38	0.45	0.41	0.04	10.0	0.629	0.641	0.635	0.059	9.3
10 s	1	0.37	0.45	0.41	0.04	9.5	0.627	0.648	0.637	0.058	9.1
	2	0.39	0.45	0.42	0.03	7.7	0.628	0.629	0.628	0.076	12.1
	3	0.37	0.45	0.41	0.04	10.0	0.619	0.647	0.633	0.088	13.9
30 s	1	0.37	0.45	0.41	0.04	9.5	0.617	0.644	0.630	0.082	13.0
	2	0.39	0.45	0.42	0.02	5.1	0.621	0.645	0.633	0.066	10.4
	3	0.38	0.45	0.41	0.03	7.3	0.623	0.648	0.635	0.089	14.0
60 s	1	0.37	0.45	0.41	0.03	6.3	0.628	0.654	0.641	0.064	9.9
	2	0.39	0.45	0.42	0.03	7.0	0.616	0.640	0.628	0.073	11.6
	3	0.37	0.44	0.41	0.04	9.8	0.622	0.645	0.633	0.071	11.2

The research results were analysed with the use of *STATISTICA 10* software on the assumed significance level of $\alpha=0.05$. In the selected sources of variation, components of variation were assumed according to the mixed model, where the time of microwave radiation (along with the control sample) was assumed as a constant factor and years of research were a random factor. Normality of distribution was determined in samples with Kolmogorov-Smirnov's test and the uniformity of variation with Levene's test. Significance of differences was investigated with the analysis of variance with the use of *F*-Snedecor's test.

Additionally, the value of Wilks' test was provided and the measure of effects size (η_p^2 was calculated – proportion of the error variation and effect explained by the effect) for the analysis of variance which was carried out. On account of the specificity of experiment (possible lack of equipotency in tests in the final stage of experiment) for multiple comparisons the Spjotvoll and Stolene's procedure was used (generalization of *HSD* Tukey's test).

Results of the experiment and discussion

Value of the coefficient of variance in samples for a variable M_f , was within 7.2-10% and for a variable φ_f within 9.1-14.3% in the first year of experiment (Table 1) in subsequent years of research analogically: M_f 7.8-10%, φ_f 11.4-14.1% and M_f 8.4-9.9%, φ_f 12.3-14.8%. Calculated values of Kolmogorov-Smirnov's statistics were insignificant (on the assumed level of $\alpha=0.05$), which means that samples accepted for the research fitted with its empirical distribution into the standard regular distribution. Doses of microwaves which were used to radiate bean seeds were within (average for the period of research): respectively for the exposition (10 s) 2.38-2.44 kJ·g⁻¹, (30 s) 6.98-7.50 kJ·g⁻¹ and (60 s) 13.95-14.63 kJ·g⁻¹. After the process of germination, between replications within the combination (in a given year when the experiment was carried out) no significant differences in the values of the investigated dependent variable (fresh mass) were reported. The analysis of variance, which was carried out, showed a significant impact of quality predictors on the investigated dependent variables (Table 2). In the next step, for statistically significant grouping variables, post-hoc tests were carried out (Tables 3-5). Due to the fact that all years of research differed significantly, further analysis (post-hoc tests) was carried out as a one-factor (treating individually each year of experiment).

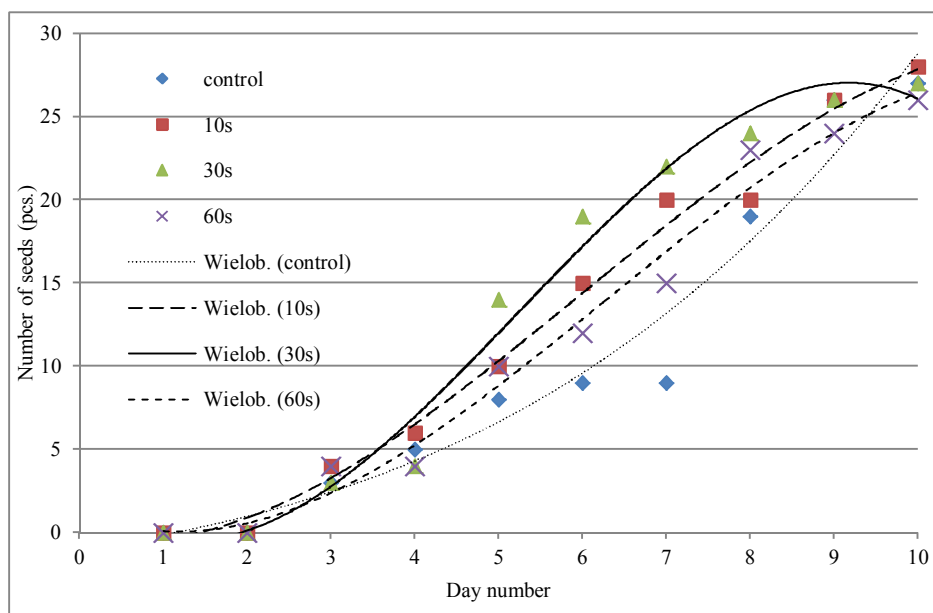


Figure 1. Number of bean seeds which germinate correctly in particular days (example for 2012)

The investigated process of germination of bean seeds, described by the number of seeds which correctly germinate (fig. 1) is characteristic for the plants of a species used in the experiment (Kornarzyński and Pietruszewski, 2008) and arranged acc. to a characteristic sigmoidal curve. In each year of research it was found out that the highest germination ability was in case of bean plants which grew out of seeds, which were radiated with microwaves for 10 s and the lowest radiation for 60 s. Bean plants which grew out of seeds, which were radiated for 10 s had a germination ability higher by 2.2% in 2012, 1.3% in 2013 and 2.5 % in 2014 in comparison to the control sample (fig. 2).

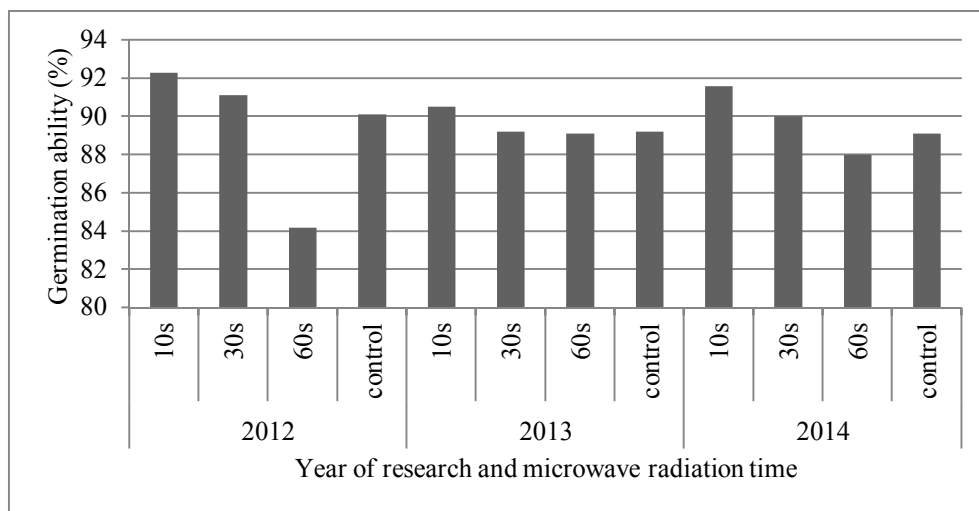


Figure 2. Germination ability of bean seeds in particular combinations of experiment

In each year of realization of the experiment it was found out that samples radiated within 10 and 30 s germinated faster with reference to the sample radiated for 60 s and to the control sample (Fig. 3). In the first case, Maguier's index assumed values within 6.2-6.4 and in the second one 5.9-6.0. In case of the evaluation of the average time of germination of one bean seed, the lowest values of Pieper's index for seeds radiated within 10 s and the highest in the control sample were reported. Interpretation of the value of this index is as follows: the lower the value the higher liveness of a seed and faster germination, the higher value the longer the germination process. Data presented in Fig. 4 show, with reference to the control (=100%), that 10 s exposition of bean seeds to microwaves caused the increase of the average germination time by 2.9%.

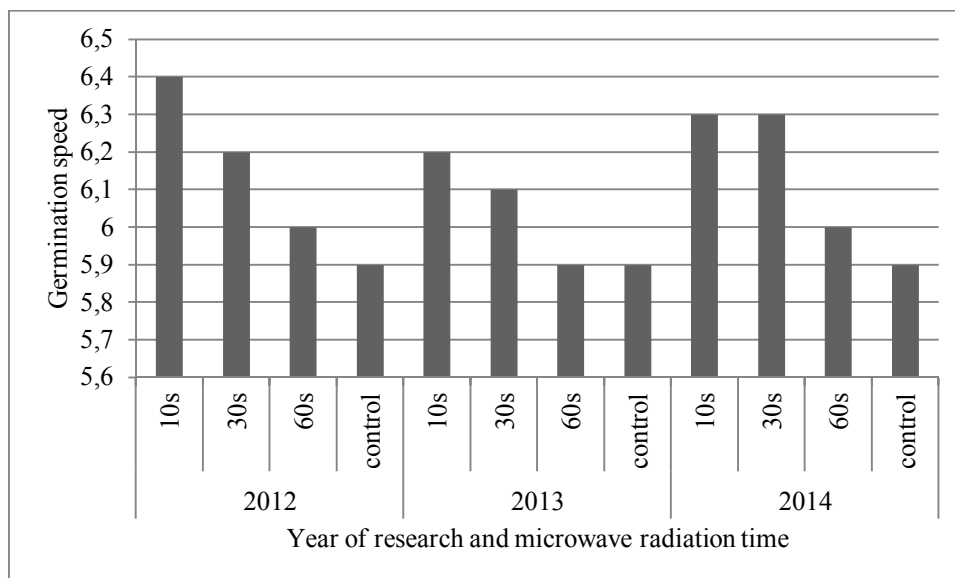


Figure 3. Relative speed of germination of bean seeds in particular combinations of experiment

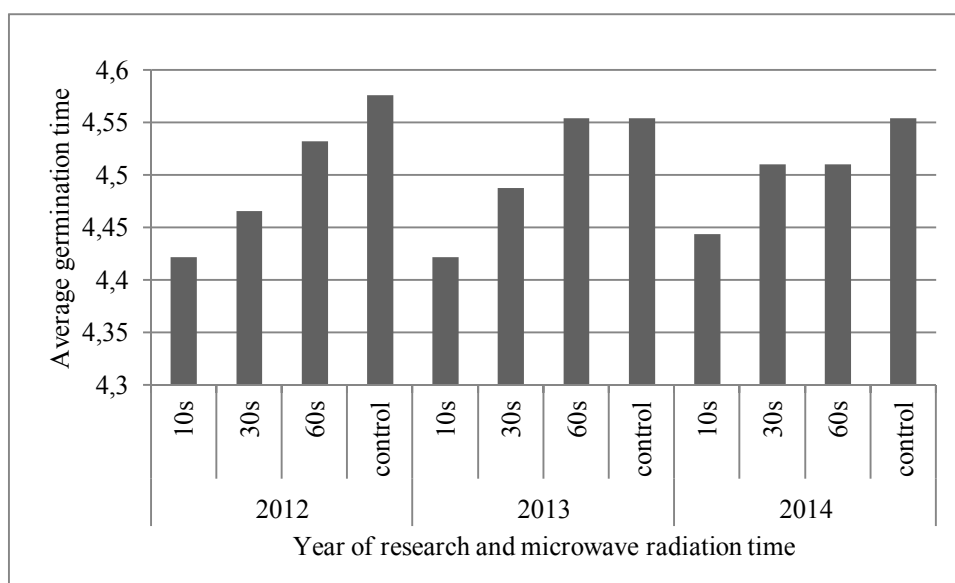


Figure 4. Average time of germination of bean seeds in particular combinations of experiment

Table 2

Result of analysis of variance; impact of research years and radiation of bean seeds with microwaves on the increase of fresh mass of a plant

Quality predictor	Test value of F -Snedecor	Probability test value	Wilks test value	η_p^2
Absolute term	23834.685	0.000	0.007	0.982
Year	13.511	0.000	0.792	0.524
Mf	12.945	0.003	0.812	0.262

Although, the analysis of variance proved significant impact of microwaves on shaping bean plant biomass (significant value $F = 12.9$) the value, calculated for this effect (a quality predictor) η_p^2 was only 0.262 (Table 2). It proves that only approximately 26.2% of the entire variation of the experiment results (biomass increase) may be explained by the impact of microwaves on the bean plant. Despite a low value of the coefficient η_p^2 which describes the size of the microwaves' effect, the quality predictor should be included, since according to the Cohen's scale (Stanisz, 2007) effects below the value of $\eta_p^2 = 0.2$ are insignificant.

Table 3

Groups in uniform average (Spjotvoll-Stoline's test); impact of radiation of bean plants with microwaves on the increase of fresh plant mass (results of research from 2012)

Microwaves radiation time (s)	Fresh mass (g)	Uniform groups	
		1	2
60	7.61	****	
30	7.75		****
10	7.81		****
Kontola	7.83		****

Table 4

Groups in uniform average (Spjotvoll-Stoline's test); impact of radiation of bean plants with microwaves on the increase of fresh plant mass (results of research from 2013)

Microwaves radiation time (s)	Fresh mass (g)	Uniform groups		
		1	2	3
60	7.49	****		
30	7.64		****	
(control)	7.71		****	
10	7.86			****

Table 5

Groups in uniform average (Spjotvoll-Stoline's test); impact of radiation of bean plants with microwaves on the increase of fresh plant mass (results of research from 2014)

Microwaves radiation time (s)	Fresh mass (g)	Uniform groups	
		1	2
60	7.52	****	
30	7.74		****
(control)	7.78		****
10	7.82		****

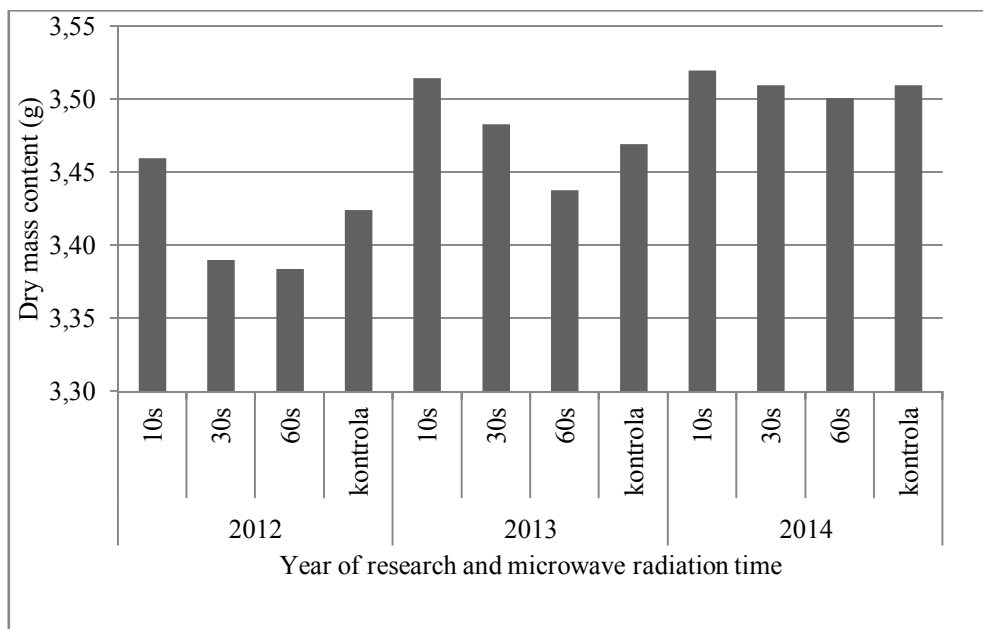


Figure 5. The content of air dry mass in bean plants in particular combinations of the experiment

Visible differences with reference to the control sample in the content of dry mass in bean plants were reported in case of seeds radiated with microwaves within 10 and 60 s. In case 10 s exposition, the content of dry mass was higher at the average by 2.9% and in case of 60 s exposition it was lower by 1.7%. The loss of plant mass as a result of water evaporation (drying) was within 44-46% (Fig. 5). Germination ability at the level of 90-92% obtained after 10 s exposition of bean seeds to microwaves, is higher than the values provided by Szafirowska (2013). This author (Szafirowska, 2013) when comparing a traditional and ecological method of bean cultivation (Aura, Augusta, Igołomska and Wawelska variety) obtained for Igołomska variety the germination ability at the level of 84-85% (germination energy 78-79%, sprouting 74-76%) – these results concern field research thus conditions, when plant is exposed to a more numerous contact with pests in comparison to a laboratory facility. According to Sinh et al., (1999) bean seeds are colonized by a numerous mykoflora (*Fusarium avenaceum*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Rhizopus nigricans*, *Fusarium* spp., *Botrytis cinerea* or *Fusarium oxysporum*) and saprophytic fungi (*Penicillium* spp., *Mucoraceae*). Wondolowska-Grabowska (2003) claims the same and states at the same time that in case of common bean seeds, this mykoflora is represented the most numerously by *Penicillium notatum*, *Fusarium avenaceum*, *Rhizopus arrhizus* and filamentous colonies. It may be assumed that in case of exposition of bean seeds to microwave radiation (100 W and 2450 MHz) identical reactions appeared as described by Tylkowska et al. (2010) and Friesen et al. (2014) with reference to mykoflora, where in similar experiments radiation with frequency of 2450 MHz was applied and a magnetron operated with the power of 650 W and 1100 W. In the above quoted research,

the authors (Tylkowska et al., 2010; Friesen et al., 2014) confirmed the decrease of population of *Penicillium* spp. and *Colletotrichum lindemuthianum* after seeds irradiation. The research carried out by Krupa and Soral-Śmietana (2005), the objective of which was to determine the impact of hydrothermal factors (inter alia microwave heating) on the enzymatic availability of protein selected from bean seeds of Aura (*Phaseolus vulgaris*) and Eureka (*Phaseolus coccineus*) variety proves that microwave radiation causes decrease of the total protein content and decrease of the amount of total mineral compounds (analysed as the ash content). Thus, there is a probability that 60 s radiation time, could cause unfavourable changes in aminoacids structure translating into an initial stage of plant development. The authors quoted above (Krupa and Soral-Śmietana, 2005) do not confirm that microwaves caused significant changes of starch content in bean seeds. According to the author, following experiments concerning the microwaves impact on the bean plants, should include subsequent varieties and the radiation time may be narrowed to 30 s.

Conclusions

1. A positive impact of 10 s radiation of bean seeds on the investigated parameters, which determine the sowing value of bean seeds (germination ability, Maguier's index, Piper's index) were confirmed.
2. Statistically significant differences in the increase of fresh mass between combinations of microwave radiation and the control sample were reported.
3. In all years of research, statistically significant negative impact of 60 s microwave exposition of bean seed on the increase of fresh mass of plants was determined.
4. In 2013, a statistically significant impact of 10 s microwave exposition of bean seeds on the increase of fresh plant mass was reported.

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OCENA WPŁYWU PRZEDSIEWNEJ MIKROFALOWEJ STYMULACJI NASION FASOLI NA PROCES ICH KIEŁKOWANIA

Streszczenie. Celem pracy była ocena wpływu stymulacji mikrofalowej nasion fasoli na proces ich kiełkowania. Badania laboratoryjne prowadzono w latach 2012-2014 z wykorzystaniem kwalifikowanych nasion fasoli (*Phaseolus vulgaris* L.) odmiany Igołomska. Bezpośrednio przed siewem nasiona napromieniowano mikrofalami przez czas 10, 30 i 60 s. Źródłem mikrofal był magnetron działający z mocą 100 W i wytwarzający fale o częstotliwości 2,45 GHz. Zobrazowano przebieg procesu kiełkowania oraz wyznaczono parametry określające wartość siewną nasion fasoli: zdolność kiełkowania, względną szybkość kiełkowania (wskaźnik Maguiera) oraz średni czas kiełkowania (wskaźnik Piepera). Określono świeżą a następnie suchą masę roślin. Uzyskane wyniki pozwalają na stwierdzenie, że mikrofały modyfikują proces kiełkowania nasion fasoli.

Słowa kluczowe: mikrofały, fasola, kiełkowanie