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EXPERIMENTAL PAPER

The effect of colour grading of milk thistle (*Silybum marianum* (L.) Gaertn.) seeds on their quality for sowing

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Summary

Introduction: Milk thistle (*Silybum marianum* (L.) Gaertn.) is a medicinal plant belonging to Asteraceae family. Extract from milk thistle achenes (termed in practice as seeds) contains sylimarin, which protects liver cells against inorganic and organic toxic compounds. **Objective:** The aim of the research was to evaluate the effect of colour grading on the quality of milk thistle seeds. **Methods:** Seeds were graded manually by colour according to the Royal Horticultural Society Colour Chart, issued in Great Britain. In three samples two fractions of seeds were separated: beige and brown, whereas seeds of the fourth sample were graded into three fractions: yellow, beige and brown. The 1000-seed weight and germination of graded and non-graded seeds were evaluated. Infestation of graded seeds with fungi was assessed. **Results:** Brown seeds had a higher 1000-seed weight than beige or yellow ones. Germination at the final count of beige seeds did not differ as compared to that of brown seeds or was even better. Milk thistle seeds were infested with numerous fungi, however *Alternaria alternata* and *Ulocladium consortiale* predominated. **Conclusions:** Less mature beige seeds can be used as sowing material because their germination at the final count did not differ as compared to that of fully

mature brown seeds or was even better. Infestation of these seeds with some of the fungi was lower than brown seeds.

Key words: *milk thistle, 1000-seed weight, seed germination, seed health*

INTRODUCTION

Milk thistle (*Silybum marianum* (L.) Gaertn.) is an important medicinal plant belonging to *Asteraceae* family; native to the Mediterranean region and the Caucasus. In Europe, milk thistle is commercially cultivated for fruits – achenes (*Silybi mariani fructus*), termed in practice as the seeds. They contain sylimarin – a complex of flavonolignans, which is used in the treatment of liver diseases such as jaundice, bile stones, hepatitis and steatosis [1]. Dietary supplements containing the extract of milk thistle fruit are the best-selling plant pharmaceuticals in the United States and Europe. Poland is one of the most important producers of raw material and medicinal preparations based on extract of milk thistle fruit in Europe [2, 3].

Flower head development in milk thistle starts with central inflorescence and it continues on primary and secondary branches. Milk thistle is harvested mechanically when 25% of inflorescences are mature. Hence, the determination of the most appropiate time of harvest is an important but difficult element of cultivation practice. A variation in seed colour during harvest occurs very often because of uneven flowering and seed ripening [4, 5]. Dyduch and Najda [6] found that brown seeds had a higher content of flavonolignans than beige and light brown seeds. The greatest content of silymarin in fully mature seeds confirmed also Martinelli *et al*. [4], Carrier *et al.* [5], Martin *et al.* [7] and Elwekeel *et al.* [8].

Seed colour is genetically controlled, but it is regulated by different physiological processes. It changes with seed age and deterioration of its quality [9]. It has been found that the content of anthocyanins and phenolic compounds in wheat seeds is affected by growing and environmental conditions [10]. Seed grading by colour is one of the basic methods of improving seed quality. In conventional colour sorting, immature seeds are rejected on the basis of their pale colour. Gugnani *et al.* [11] reported that blackish brown or light brown cabbage seeds possessed a much higher germination capacity than honey yellow ones. Colour sorting also improved seed quality in pansy, China aster and sesame [12-14].

The aim of the conducted research was to evaluate the effect of colour grading on the quality of milk thistle seeds.

MATERIAL AND METHODS

In the study, four seed samples of milk thistle cv. 'Silma', produced for reproduction, were used. Samples I–III were obtained from Poznańskie Zakłady

Zielarskie "Herbapol" S.A., while sample IV from Firma Zielarska M. Lewandowski. The seeds were reproduced in the area of Wielkopolskie and Kujawsko-Pomorskie voivodeships. Two-stage harvest was applied. Seeds of samples I-II were collected in 2009 and III-IV in 2010. The seeds were examined about six months after the harvest.

Seeds were graded manually by colour based on Royal Horticultural Society Colour Chart, issued in Great Britain [15]. Due to variation in the colour, seeds in individual samples were divided as follows:

1. Seed sample I:
a) beige seeds: greyed-yellow group – 161 CD, 162 D, greyed-brown group – 199 BCD
b) brown seeds: grey-brown group – 199 ABCD, brown group – 200 ABCD, black group – 202 A
2. Seed sample II:
a) beige seeds: greyed-yellow group – 160 D, 161 ACD, 162 CD, greyed-orange group – 164 B, 177 D, greyed-brown group – 199 BCD
b) brown seeds: grey-brown group – 199 AB, brown group – 200 ABC, greyed-orange group – 165 A, 166 A
3. Seed sample III:
a) beige seeds: greyed-yellow group –161 ACD, 162 CD, greyed-orange group – 174 D, 177 D, greyed-brown group – 199 D, grey group – 201 B
b) brown seeds: greyed-orange group – 165 A, grey-brown group – 199 AB, brown group – 200 ABCD, greyed-orange group – 165 A, 166 A
4. Seed sample IV:
a) yellow seeds: greyed-yellow group –161 BCD, 162 A, greyed-orange group – 164 DC
b) beige seeds: greyed-yellow group –161 A, greyed-green group – 197 B, grey-brown group – 199 BCD
c) brown seeds: greyed-orange group – 165 AB, greyed-orange group – 174 B, 177 A, greyed-brown group – 199 A, brown group – 200 ABC, black group – 202 A.

The weight of 1000 seeds and germination at the first and final counts were evaluated for graded and non-graded (control) seeds according to International Seed Testing Association (ISTA) rules [16]. Moreover, in individual samples the percentage share by weight of seeds from separated fractions was determined.

Germination test was conducted on four replicates of 100 seeds from each fraction or non-seperated control. Seeds were placed in 9 cm Petri dishes containing six layers of blotting paper wetted with distilled water and incubated at 20°C in darkness for 21 days. After 7 days germination at the first count was determined, whereas after 21 days germination at the final count, the percentages of abnormal diseased and deformed seedlings, fresh and dead seeds were evaluated.

Mycological analysis was performed on 200 seeds (four replicates of 50) from each fraction by using the deep-freezing blotter test. Seeds were incubated in Petri dishes on six layers of moist blotting paper, 10 per dish, for two days at 20°C in darkness, then transferred at -20°C for 24 h and subsequently incubated for seven days at 20°C under alternating cycles of 12 h NUV light and 12 h darkness. The

fungi were identified on the basis of their growth and sporulation characteristics using a stereomicroscope and a compound microscope [17-19].

The obtained results were evaluated by means of variance analysis followed by the Duncan's multiple range test at α=0.05. The analysis was performed using STAT software.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

In samples I, II and III two fractions of seeds were separated: beige and brown. The percentage share of beige seeds ranged from 17.0 to 35.8. They were characterized by a lower 1000-seed weight than brown and non-graded seeds. In sample III the 1000-seed weight of brown seeds was significantly higher compared to control (tab. 1). Seeds of sample IV were graded into three fractions: yellow, beige and brown. The biggest percentage share was recorded for beige seeds, whereas the smallest in case of brown seeds. Yellow seeds had a lower 1000-seed weight than beige, brown and non-graded seeds. Brown seeds showed the greatest value of this parameter (tab. 2).

Table 1.

1000-seed weight (TSW) and share of colour fractions in samples I, II and III

Seed sample	Non-graded seeds (control)	Beige seeds		Brown seeds	
	TSW [g]	TSW [g]	share in seed sample [weight %]	TSW [g]	share in seed sample [weight %]
I	24.0 b	22.7 a	35.8	24.0 b	64.2
II	26.9 b	24.4 a	17.0	27.3 b	83.0
III	26.5 b	23.8 a	17.0	27.4 c	83.0

Means in rows followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Table 2.

1000-seed weight (TSW) and share of colour fractions in sample IV

Non-graded seeds (control)	Yellow seeds		Beige seeds		Brown seeds	
TSW (g)	TSW [g]	share in seed sample [weight %]	TSW (g)	share in seed sample [weight %]	TSW [g]	share in seed sample [weight %]
29.8 b	26.9 a	39.1	30.0 b	46.9	30.9 c	14.0

Means followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Milk thistle seed germination at the first and final counts in all examined samples was low because of the high percentage of diseased seedlings and dead seeds (tab. 3–6). In sample I, germination at the first count of beige seeds was significantly higher than brown and non-graded seeds. Beige seeds of sample II also germinated better than brown and non-graded seeds. They showed the greatest germination at the first and final counts and the lowest percentage of diseased seedlings. In both samples germination at the first count of brown seeds was the worst (tab. 3 and 4). On the contrary, brown seeds of sample III were characterized by a higher value of this parameter than beige and non-graded seeds (tab. 5). In sample IV germination at the first and final counts of seeds from different fractions and control did not vary significantly. However, in case of brown seeds a lower percentage of diseased seedlings and a higher percentage of dead seeds compared with yellow, beige and non-graded seeds were found (tab. 6).

Table 3.

Germination of non-graded and colour graded seeds in sample I

	Germination at the first count [%]	Germination at the final count [%]	Abnormal seedlings		Fresh seeds [%]	Dead seeds [%]
			diseased [%]	deformed [%]		
C*	17.8 b	39.3 a	48.8 a	1.8 b	0.3 a	10.3 a
B	25.0 c	42.3 a	44.8 a	1.3 b	0 a	11.3 a
BR	10.0 a	36.3 a	55.0 a	0.3 a	0.3 a	8.0 a

* C – non-graded seeds (control), B – beige seeds, BR – brown seeds,
Means in columns followed by the same letter are not significantly different at α=0.05 level according to Duncan's multiple range test.

Table 4.

Germination of non-graded and colour graded seeds in sample II

	Germination at the first count [%]	Germination at the final count [%]	Abnormal seedlings		Fresh seeds [%]	Dead seeds [%]
			diseased [%]	deformed [%]		
C*	14.0 b	41.0 ab	51.8 b	0.3 a	1.8 a	5.3 a
B	22.3 c	50.3 b	38.8 a	1.8 a	3.3 a	6.0 a
BR	7.8 a	32.8 a	62.0 b	1.0 a	1.8 a	2.8 a

*C – non-graded seeds (control), B – beige seeds, BR – brown seeds,
Means in columns followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Table 5.

Germination of non-graded and colour graded seeds in sample III

	Germination at the first count [%]	Germination at the final count [%]	Abnormal seedlings		Fresh seeds [%]	Dead seeds [%]
			diseased [%]	deformed [%]		
C*	23.3 a	53.0 a	32.8 a	3.3 a	1.0 ab	10.0 a
B	25.0 a	50.0 a	32.0 a	3.3 a	3.0 b	11.8 a
BR	31.8 b	52.0 a	37.3 a	1.3 a	0 a	9.3 a

* C – non-graded seeds (control), B – beige seeds, BR – brown seeds,
Means in columns followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Table 6.

Germination of non-graded and colour graded seeds in sample IV

	Germination at the first count [%]	Germination at the final count [%]	Abnormal seedlings		Fresh seeds [%]	Dead seeds [%]
			diseased [%]	deformed [%]		
C*	19.3 a	31.8 a	42.8 b	4.0 a	0 a	21.8 a
Y	19.8 a	30.0 a	46.8 b	3.0 a	0.3 a	20.0 a
B	22.8 a	37.3 a	38.3 ab	2.3 a	0 a	22.0 a
BR	17.0 a	36.3 a	28.3 a	1.8 a	0 a	33.8 b

* C – non-graded seeds (control), B – beige seeds, BR – brown seeds, Y – yellow seeds
Means in columns followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Milk thistle seeds were infested with numerous fungi: *Acremoniella atra* (Corda) Sacc., *Alternaria alternata* (Fr.) Keissler, *Alternaria* sp., *Aspergillus flavus* Link, *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Botrytis cinerea* Pers., *Cladosporium* spp., *Epicoccum nigrum* Link, *Fusarium* spp., *Melanospora simplex* (Corda) D.Hawksw., *Melanospora* sp., *Mucor* spp., *Penicillium* spp., *Phoma* sp., *Phomopsis* sp., *Rhizopus stolonifer* (Ehrenb.) Vuill, *Sordaria* sp., *Trichothecium roseum* (Pers.) Link ex S.F. Gray, *Stemphylium botryosum* Wallr., *Ulocladium consortiale* (Thüm) E. Simmons and *Verticillium* spp. Among them *A. alternata* and *U. consortiale* predominated (tab. 7-10). In sample I brown seeds were infested with *A. alternata, M. simplex* and *U. consortiale* to a greater degree than beige seeds. Higher infestation of brown seeds with *U. consortiale* was observed also in sample II. *Cladosporium* spp. and non-sporulating mycelium were detected more frequently on brown seeds of sample IV than on yellow and beige ones. Only in sample III the incidence of non-sporulating mycelium on beige seeds was higher than on brown seeds (tab. 7-10).

Table 7.

Incidence of fungi on colour graded seeds in sample I (percentage of infested seeds)

Fungus	B*	BR
Acremoniella atra	1.0 a	0 a
Alternaria alternata	49.5 a	64.0 b
Alternaria sp.	0 a	0.5 a
Aspergillus flavus	0 a	0.5 a
Bipolaris sorokiniana	0.5 a	0 a
Cladosporium spp.	3.5 a	7.0 a
Epicoccum nigrum	1.5 a	5.0 a
Fusarium spp.	3.5 a	6.0 a
Melanospora simplex	3.0 a	11.5 b
Mucor spp.	0.5 a	1.0 a
Penicillium spp.	1.5 a	3.5 a
Phoma sp.	1.0 a	0 a
Stemphylium botryosum	0.0 a	1.0 a
Ulocladium consortiale	46.5 a	61.5 b
Verticillium spp.	25.5 a	17.5 a
Non-sporulating mycelium	6.5 a	3.5 a
Seeds free of fungi (%)	14.0 a	7.5 a

* B – beige seeds, BR – brown seeds,
Means in rows followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Table 8.

The incidence of fungi on colour graded seeds in sample II (the percentage of infested seeds)

Fungus	B*	BR
Alternaria alternata	36.5 a	26.5 a
Aspergillus flavus	0 a	1.0 a
Botrytis cinerea	0.5 a	2.5 a
Cladosporium spp.	2.0 a	4.5 a
Epicoccum nigrum	3.0 a	3.0 a
Fusarium spp.	4.0 a	4.0 a
Melanospora sp.	0.5 a	0 a
Penicillium spp.	5.0 a	2.5 a
Phoma sp.	0.5 a	0.5 a
Rhizopus stolonifer	1.0 a	0 a
Stemphylium botryosum	1.0 a	0 a

Fungus	B*	BR
Ulocladium consortiale	14.5 a	28.5 b
Verticillium spp.	0.5 a	3.0 a
Non-sporulating mycelium	8.5 a	6.0 a
Seeds free of fungi (%)	34.5 a	35.0 a

* B – beige seeds, BR – brown seeds,
Means in rows followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Table 9.

The incidence of fungi on colour graded seeds in sample III (the percentage of infested seeds)

Fungus	B*	BR
Alternaria alternata	26.0 a	23.5 a
Botrytis cinerea	0 a	0.5 a
Cladosporium spp.	2.0 a	4.0 a
Epicoccum nigrum	1.0 a	0.5 a
Fusarium spp.	3.0 a	1.5 a
Mucor spp.	6.5 a	6.5 a
Penicillium spp.	1.0 a	1.0 a
Stemphylium botryosum	0.5 a	2.0 a
Sordaria sp.	0 a	0.5 a
Ulocladium consortiale	25.0 a	33.5 a
Verticillium spp.	4.0 a	3.5 a
Non-sporulating mycelium	19.0 b	6.0 a
Seeds free of fungi (%)	31.5 a	35.5 a

*B – beige seeds, BR – brown seeds,
Means in rows followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

Table 10.

The incidence of fungi on colour graded seeds in sample IV (the percentage of infested seeds)

Fungus	Y*	B	BR
Acremoniella atra	0.5 a	3.0 a	1.5 a
Alternaria alternata	90.0 a	91.0 a	95.0 a
Alternaria spp.	1.5 a	1.0 a	5.0 a
Aspergillus spp.	0.5 a	0 a	0 a
Cladosporium spp.	29.0 a	32.0 a	44.0 b
Epicoccum nigrum	1.5 a	1.5 a	1.5 a
Fusarium spp.	10.0 a	10.0 a	5.0 a
Mucor spp.	12.5 a	9.5 a	8.5 a
Penicillium spp.	3.0 a	1.0 a	1.0 a

Fungus	Y*	B	BR
Phomopsis sp.	0 a	0 a	0.5 a
Rhizopus stolonifer	0 b	2.0 a	0 b
Stemphylium botryosum	3.0 a	2.0 a	2.0 a
Trichothecium roseum	0 a	2.0 a	1.0 a
Ulocladium consortiale	22.5 b	38.5 a	34.0 a
Verticillium spp.	6.5 a	11.5 a	10.5 a
Non-sporulating mycelium	12.0 c	31.0 b	44.5 a

* Y – yellow seeds, B – beige seeds, BR – brown seeds,
Means in rows followed by the same letter are not significantly different at α=0.05 level, according to Duncan's multiple range test.

DISCUSSION

Milk thistle seed colour of the examined samples varied and depended on seed maturity. In three samples, beige and brown seeds were graded, whereas in case of fourth sample three fractions were separated: yellow, beige and brown seeds. Elwekeel *et al.* [8] reported that the colour of milk thistle seeds changed during maturation from creamy white to dark brown when they were fully mature. It has been found that brown seeds, fully mature, had a higher 1000-seed weight than beige or yellow ones. The percentage share of brown seeds varied to a large extent among the examined samples. Dyduch and Najda [6] graded milk thistle seeds by colour into three fractions: beige, light brown and dark brown. They observed the highest 1000-seed weight in case of fully mature seeds – dark brown. Czabajska *et. al*. [20] also reported a higher 1000-seed weight for dark milk thistle seeds than for light ones. According to the authors weather conditions during seed development affect the percentage share of light seeds in a total yield. Warm, sunny weather with low rainfalls favour the production of dark seeds. The authors found that during cold and humid weather a share of light seeds reached even 70%.

Milk thistle seed germination at the first and final counts in all examined samples was low because of the high percentage of diseased seedlings and dead seeds. Health analysis showed the presence of numerous fungi. The predominant species was *A. alternata*, which in opinion of Cwalina-Ambroziak *et al.* [21] is a potential pathogen of milk thistle. Machowicz-Stefaniak and Zimowska [22] and Rosińska *et al.* [23] also detected *A. alternata* on milk thistle seeds very frequently, even after seed disinfection what confirms its presence also in inner tissues. Tylkowska [24] observed the reduction in carrot seed germination and the increase in the numbers of diseased seedlings and dead seeds after inoculation of disinfected carrot seeds with this fungus. Nowicki [25] found that about 30% of *A. alternata* isolates, obtained from carrot seeds, showed a weak pathogenicity to seedlings of this species. Isolates obtained from infected carrot seedlings were mostly pathogenic [26]. *Alternaria alternata* is capable of producing several mycotoxins, such as alternariol,

alternariol monomethyl ether, altenuene, altertoxins I, II, III and tenuazonic acid [27, 28]. Bottalico and Logrieco [29] reported that tenuazonic acid exhibited a severe growth inhibiting activity both on root and on the shoot of germinating tomato seeds. The phytotoxic effect was highly correlated to tenuazonic acid concentration. According to Cwalina-Ambroziak *et al.* [21] *B. cinerea* and fungi of the genera *Fusarium* and *Phoma* are potential pathogens of milk thistle, too. They were identified in the examined seed samples, but less frequently than *A. alternata*. Machowicz-Stefaniak and Zimowska [22] detected on milk thistle seeds *Fusarium avenaceum* (Fr.) Sacc. (current name *Gibberella avenacea* R. J. Cook) and *F. sporotrichioides* Sherb. The negative effect on seed germination can have also saprotrophic fungi, such as *Aspergillus* spp. and *Penicillium* spp., which produce secondary metabolites (mycotoxins) and amylolytic, proteolytic and lipolytic enzymes [30].

It has been found that germination and health of seeds from different fractions varied among samples. In samples I and II, beige seeds showed greater germination at the first count than brown and non-graded seeds. Moreover, in case of the latter a higher germination at the final count and a lower percentage of diseased seedlings were found. Brown seeds of sample I were infested with *A. alternata*, *M. simplex* and *U. consortiale* to a larger degree than beige seeds, whereas in sample II *U. consortiale* was detected more frequently on brown seeds. In sample IV, germination between graded and non-graded seeds did not differ significantly, however the highest percentage of dead seeds was recorded in the fraction of brown seeds, which were infested with *Cladosporium* spp. and non-sporulating mycelium to the largest extent. Only in sample III brown seeds showed better germination at the first count than beige and non-graded seeds. The incidence of non-sporulating mycelium on these seeds was lower than on beige seeds. Dyduch and Najda [6] found the highest germination at the first and final counts for light brown seeds followed by dark brown ones. They reported that beige seeds showed poorer germination than non-graded seeds. On the contrary Czabajska *et al.* [20] did not find the effect of seed colour on germination. However, in some cases the authors observed faster emergence after sowing of light seeds.

Based on the obtained results it can be concluded that less mature beige seeds can be used as sowing material because their germination at the final count did not differ as compared to that of fully mature brown seeds or was even better. Infestation of these seeds with some of the fungi was lower than brown seeds.

CONCLUSIONS

1. Brown seeds had a higher 1000-seed weight than beige or yellow ones.
2. Germination at the final count of less mature, beige seeds did not differ as compared to that of fully mature brown seeds or was even better.
3. Infestation of beige seeds with some of the fungi was lower than brown seeds.

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WPŁYW FRAKCJONOWANIA NASION OSTROPESTU PLAMISTEGO (SILYBUM MARIANUM (L.) GAERTN.) POD WZGLĘDEM BARWY NA ICH WARTOŚĆ SIEWNĄ.

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S t r e s z c z e n i e

Wstęp: Ostropest plamisty (*Silybum marianum* (L.) Gaertn.) jest rośliną leczniczą należącą do rodziny *Asteraceae*. Wyciąg z niełupek ostropestu (w praktyce zwanych nasionami) zawiera sylimarynę, która chroni komórki wątroby przed szkodliwym działaniem substancji organicznych i nieorganicznych. **Cel:** Celem badań było określenie wpływu frakcjonowania nasion pod względem barwy na ich jakość. **Metody:** Nasiona podzielono ręcznie na frakcje różniące się barwą na podstawie katalogu barw wydanego przez Królewskie Towarzystwo Ogrodnicze w Wielkiej Brytanii. W trzech próbach wydzielono dwie frakcje nasion: beżowe i brązowe, natomiast nasiona próby czwartej podzielono na trzy frakcje: żółte, beżowe i brązowe. Oceniono masę 1000 nasion oraz kiełkowanie nasion frakcjonowanych i nie-

frakcjonowanych. Określono też występowanie grzybów na nasionach frakcjonowanych. **Wyniki:** Nasiona brązowe charakteryzowały się większą masą 1000 nasion niż beżowe lub żółte. Zdolność kiełkowania nasion beżowych nie różniła się lub była lepsza od zdolności kiełkowania nasion brązowych. Nasiona ostropestu były zasiedlone przez liczne grzyby, jednak dominowały *Alternaria alternata* i *Ulocladium consortiale*. **Wnioski:** Nasiona beżowe, mniej dojrzałe, mogą być wykorzystane jako materiał siewny, ponieważ ich zdolność kiełkowania nie różniła się lub była nawet lepsza od zdolności kiełkowania w pełni dojrzałych nasion brązowych. Zasiedlenie tych nasion przez niektóre grzyby było mniejsze niż nasion brązowych.

Słowa kluczowe: *ostropest plamisty, masa 1000 nasion, kiełkowanie nasion, zdrowotność nasion*