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EFFECT OF TEMPERATURE DECREASE ON AESCINE CONTENT IN SEEDS OF WHITE HORSE-CHESTNUT (*AESCULUS HIPPOCASTANUM* L.)

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INTRODUCTION

One of the main tasks of botanical gardens is botanical acclimatization and introduction of plants for scientific, educational, and cognitive purposes (Łukasiewicz A. 2003). Tracing the development of plants can be a basis for further investigations, e.g., biometric, physiological, including chemical composition of leaves, seeds, etc. However, despite complying with the methodological requirements such as sample representativeness or a proper number of replications, during plant material acquisition there might be external conditions which substantially influence the analysis results. Thus, in order to obtain reliable results, we should pay particular attention to the acquisition of the plant material for further research. For example, definite ripening and fall of white horse-chestnut seeds usually takes about three weeks, until the beginning of October. During this period, the temperature of air and soil clearly decreases, and the first ground frost occurs.

For many years large amounts of *Aesculus hippocastanum* L. seeds were exported from the area of Słupsk (Poland) for the pharmaceutical industry in Germany. In the 1980s a few railway cars of these seeds were returned to the Polish contractor due to their too low aescine content. It turned out that problems with storing and drying the seeds delayed their shipment to the pharmaceutical companies until November, which included the period of autumn ground frosts. A similar effect of aescine content drop resulting from low temperatures was observed independently of the above mentioned incident, in the seeds collected in the area of Nowa Huta, Poland (Kmieć, unpublished data).

GOAL OF THE RESEARCH

The goal of the research was to explain the influence of low temperatures on the aescine content in the seeds of white horse-chestnut *Aesculus hippocastanum* L. In 1980s, the effect of lowering its content in the seeds could be observed, simultaneously with temperature lowering. The research done in 1996 and 1999 confirmed this trend, providing additional information about aescine content changes at the temperature of –2°C.

MATERIALS AND METHODS

In 1996 fresh, ripe seeds of white horse-chestnut were collected from 22 trees in the area of parks and gardens of Cracow right after their fall, in the second weeks of September, about 50 seeds from each tree. The seeds were divided into three groups, i.e.:

K – control. The seeds of this group were dried at room temperature for three months.

SM – dried frozen. The seeds were dried like the control group, and next they were stored for 30 days in paper bags in the temperature of –8°C.

MM – fresh frozen. The fresh, wet seeds right after harvesting were placed in paper bags and stored in the temperature of –8°C for 30 days. After this period the seeds were dried like the control group. In all grups damaged seeds were removed.

The purpose of differentiating the seeds into dry and fresh in the first year of the research was to determine whether the water contained in the fresh seeds affects the aescine content rate (Table 1).

Table 1. The decrease of content of aescine in dried and fresh seeds of white horse-chstnut *Aesculus hippocastanum* L.

Tree number	Aescine content in seeds [%]			Decrease of content [%] K = 100	
	Control K	SM Dry frozen	MM Fresh frozen	SM/K	MM/K
1	12,55	9,20	8,15	–26,69	–35,06
2	7,62	6,11	6,99	–19,82	–8,27
3	7,68	7,36	6,19	–4,17	–19,40
4	15,49	10,83	10,95	–30,08	–29,31
5	8,50	8,22	7,07	–3,29	–16,82
6	10,91	9,09	7,22	–16,68	–33,82
7	7,26	6,69	6,77	–7,85	–6,75
8	8,63	9,09	5,74	5,33	–33,49
9	13,22	9,83	9,22	–25,64	–30,26
10	12,96	12,53	11,69	–3,32	–9,80
11	11,71	11,87	9,20	1,37	–21,43
12	8,93	8,85	8,15	–0,90	–8,73
13	10,34	9,97	8,44	–3,58	–18,38
14	10,08	10,83	8,24	7,44	–18,25
15	9,24	8,44	6,23	–8,66	–32,58
16	8,73	10,14	8,46	16,15	–3,09
17	10,20	10,32	7,24	1,18	–29,02
18	9,23	8,85	7,93	–4,12	–14,08
19	8,93	7,68	7,13	–14,00	–20,16
20	9,97	11,06	8,15	10,93	–18,25
21	8,15	7,13	5,33	–12,52	–34,60
22	9,01	10,59	7,69	17,54	–14,65
Average	9,97 ±2,09	9,30 ±1,68	7,83 ±1,53	–5,52 ±13,11	–20,74 ±10,11
P>F	****	****	****	ns	***

≤0.001; *≤0.0001; ns – non significanto

In 1999 about 5 kg of freshly fallen seeds were collected from two trees. As in 1996, damaged seeds were sorted out (overdried, with one flat surface, etc.). The material acquired in this way was exposed to lowered temperatures, with varying storage time from 2 to 30 days. The temperature range was also increased from +6 through –2 down to –12°C (Table 2). The seeds were divided into portions of a few seeds so that each variant (temperature*number of days) could be repeated three times. Therefore, the aescine contents presented in Tables 1 and 2 are arithmetic means out of three measurements, for each variant.

In both years of the research, the analyses were done directly after terminating the freezing.

In the first year, 1996, the experiment was to confirm or to reject the assumption about the substantial influence of temperature drop on the aescine content in the seeds. In the successive year of the experiments, 1999, the temperature range included: cooling (+6°C) and a full range of autumn ground frosts (–1, –2, –3, –4, –5°C) to first frosts (–8, –12°C). In this work, in order to make the figures clear, the author has chosen results representative for the whole temperature range, i.e. +20, +6, –2, –12°C.

In order to determine the aescine content of the chestnut seeds, the authors used the colorimetric method described by Schlemmer (1966) using ferric chloride in the presence of concentrated sulfuric acid and glacial acetic acid. Aescine was extracted with methanol from powdered and derinded seeds (sieve of 0.3 mm).

RESULTS

A substantial difference aescine content was observed in 1996, between the control group and frozen seeds, both after previous drying and

Table 2. Percent decrease content of aescine in seeds of white horse-chestnut *Aesculus hippocastanum* L. According to temperature decrease [°C]. K – control

Tree number	Freeze time [days]	Temperature (°C) Control +20	+6	–2	–12
1	2	8,80	8,86	7,57	9,42
2	2	9,85	9,50	9,20	8,81
1	4	8,80	8,91	7,81	7,16
2	4	9,85	9,4	8,09	8,65
1	6	8,80	8,33	7,73	6,09
2	6	9,85	9,01	8,03	6,89
1	8	8,80	6,68	7,59	6,63
2	8	9,85	9,07	8,65	6,83
1	10	8,80	8,74	7,38	7,27
2	10	9,85	8,54	8,69	8,16
1	12	8,80	8,20	7,17	6,49
2	12	9,85	8,77	9,77	7,52
1	14	8,80	8,21	7,01	7,24
2	14	9,85	10,26	9,32	12,22
1	16	8,80	8,33	6,86	7,04
2	16	9,85	9,18	9,50	8,89
1	18	8,80	8,05	7,81	6,53
2	18	9,85	9,50	10,36	8,32
1	20	8,80	8,73	7,96	7,30
2	20	9,85	10,79	9,20	9,10
1	22	8,80	8,31	6,64	5,75
2	22	9,85	9,46	9,95	7,79
1	24	8,80	8,33	8,69	5,78
2	24	9,85	10,01	8,63	8,65
1	26	8,80	7,61	7,45	7,45
2	26	9,85	10,57	8,75	6,91
1	28	8,80	8,77	8,68	7,08
2	28	9,85	8,42	10,03	7,85
1	30	8,80	8,05	7,91	6,79
2	30	9,85	10,36	9,85	7,42
Average		9,33 ±0,74	8,90 ±0,91	8,41 ±1,03	7,60 ±1,30
Decrease of average value (%)			–4,61	–9,86	–18,54

in fresh seeds, frozen immediately after harvesting (Table 1). The content of aescine in the control seeds was on average about 10%, ranging from 7 to 15%. In the seeds dried to the air-dry state and next frozen, the average aescine content was 9% with extreme values from 6 to 13%. Whereas in the group of seeds frozen right after harvesting, the aescine content dropped to 8%, ranging from 5 to 12%. With lowering temperature in the fresh seeds a substantial ($p=0.0004$) aescine content drop was observed (Table 1).

Analogous results were obtained in 1999 with fresh seeds stored in varying temperatures ranging from +6 through –2 down to –12°C (Table 2). The aescine content decreased from 9.3% (control group) to 8.9% (for +6°C) to 8.4% (for –2°C) down to 7.6% (for –12°C). The content drop of that compound in comparison to the control conditions was considerable and was from 4.6% to almost 19%. For the temperatures of +6 and –12°C, the authors did not observe changes in the aescine content when the period of freezing, was lenghtened from 2 to 30 days. Whereas for the temperature of –2°C there is a clear trend towards increase of aescine content in the seeds with prolonging the time of exposing them to the lowered temperature

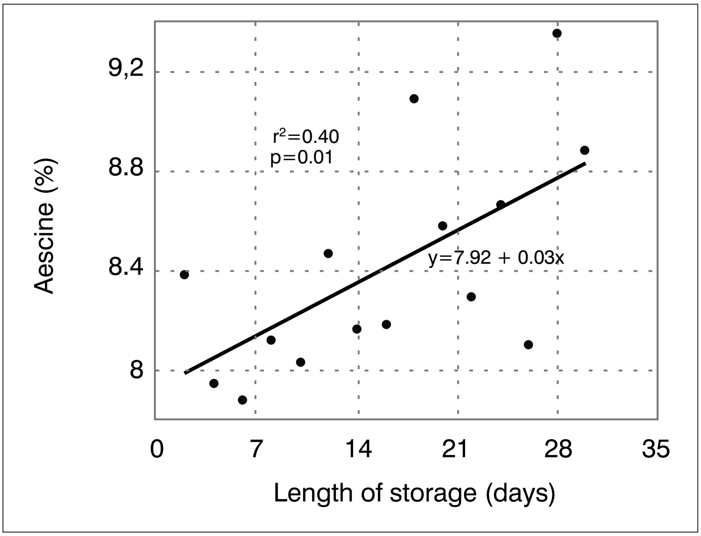

Fig. 1. Changes in aescine's contents in white horse-chestnut *Aesculus hippocastanum* L. seeds kept in a temperature of –2°C.

p=0.01 (Fig. 1). There were not, however, statistically essential differences between the seed samples coming from different trees.

DISCUSSION

The main active substance in the chestnut seeds (*A. Hippocastanum* L.), aescine, is a mixture of over 30 compounds. They are triterpenoid saponosides derived from (-amyrin, whose basic aglycones are aescigenin, protoaescigenin, and barringtogenol C (Kuhn 1963, Kuhn 1964, Tschesche 1965, Woitke 1970, Wulff 1969). In the position of atom C-3 of the aglycones, there are sugar chains made of glucuronic acid, to which in position 2 and 4, 2 glucose molecules are attached or in position 2, a glucose molecule, and in position 4, single molecules of xylose or galactose. Aglycones in the position of atom C-21 are astrified by isobutyric, α-methylobutyric, angelic, and tyglic acids, where the latter two occur most often, and at the atom C-22 they are estrified by acetic acid (Wagner et al 1970, Wulff 1969).

In therapy the whole complex of sanoposides is used internally and externally as liquid or densified extracts, ointments, jellies, or isolated pure α-aescine, or amorphic β-aescine. Its anti-inflammatory, anti-swelling, anti-effusion, sealing, and vein flexing action makes it useful in almost all branches of medicine, with many diseases. Some are very serious, life threatening, brain and lung swellings. Others are vein diseases such as hemorrhoids and varices. Sports medicine very often makes use of aescine's healing properties due to numerous injuries in many sports. The list of pharmaceuticals containing aescine is very long on the pharmaceutical market.

The aescine content in white horse-chestnut seeds is very high. According to the literature, the content ranges from 3 to 28% (Hoppe 1963, Ożarowski et al 1989, Kmieć 1977). Results obtained from the area of Poznań are between 7 and 16%. Such great content differences are caused by many factors. Correlations of aescine with parameters of urban environment show that the main factors are: the amount of free soil area around the trees, water permeability to the depth of one meter, and the organic carbon content in the substrate (Łukasiewicz, Kmieć 2003).

The results obtained in this study point out a significant effect of lowering the content of this compound in seeds exposed to low temperatures. The data of 1996 (Table 1) show that there is a substantial drop of aescine content, from 5.5% in the dried seeds to 21% in the fresh ones. This points out a significant role of the temperature*water-content interaction in the seeds on the aescine level. It is likely high water content in seeds is a factor which enables faster chemical reactions that lead to decomposition of aescine.

The research repeated in 1999 confirmed this effect. Exposing the seeds to temperatures from +6 to –12°C resulted in aescine content drop in the seeds with temperature drop. The average drop was 5, 10, 19%, respectively. An analogous process was observed also in other thermal setups (–1, –3, –4, –5, and –8°C, data not shown). Aescine decline was observed with temperature drop at all of the temperatures.

Interesting results were obtained by introducing different periods of freezing, from 2 to 30 days. It turned out that for the temperatures of +6 and –12°C, there is not a considerable difference in aescine concentration changes with time, whereas for the temperature of –2°C, there is a clear, statistically signiticant trend ($p=0.01$, Fig. 1). After the initial aescine concentration declined by 15%, we observed a steady increase of its content, with the passing exposure time, so that after 22–24 days, the content of aescine did not differ substantially from the control value. The reasons for the described course of changes in aescine content during the seed storage require further research.

CONCLUSIONS

1. The temperature drop below 10°C results in the substantial lowering of the aescine content in the seeds of white horse-chestnut *Aesculus hippocastanum* L.
2. An essential factor affecting the aescine content is the water content in the seeds while exposed to low temperatures. Our research proved that the aescine content was 6 and 21% for dried and fresh seeds, respectively, frozen at the temperature –8°C, in comparison to the control conditions (Table 1).
3. At varied thermal conditions of seed storage (+6, –2, and –12°C), the mean aescine content decline was 5, 10, 19%, respectively (Table 2).
4. In the seeds stored for 20-30 days at the temperature of –2°C, after an initial aescine level decline, it was found that the content of this compound is restituted with passing time (Fig. 1).
5. During harvesting the seeds for pharmaceutical purposes, we should pay particular attention to weather conditions while they are harvested, stored, and transported. It is particularly important to avoid exposing the seeds with high water content to low temperatures. Conditions of that kind can significantly affect their aescine content.

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