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GLUCOSINOLATES OCCURING IN GREEN PARTS OF MUSTARD (*SINAPIS ALBA* L.) AND THEIR BIOLOGICAL ACTIVITY

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Abstract. The content and composition of glucosinolates in green parts of mustard plant (*Sinapis alba* L.), at different stages of plant development were analysed. Four mustard varieties: Salvo, Metex, Ascot and Nakielska were taken under consideration. It was stated that in green parts of mustard sinalbin was dominating component of glucosinolates, but also glucotropeolin and small amounts of glucobrassicanapin apeared. The content of these compounds changes during vegetation and it is different in particular plant organs. Biological activity of mustard glucosinolates towards cabbage aphid was also studied. The correlation between the content of glucosinolates in plant and its susceptibility to aphid (*Brevicoryne brassicae* L.) infestation was checked. It was found that higher level of glucosinolates in plant caused some limitation of this pest development. Also the activity of water and alcohol extracts from mustard leaves was tested in relation to cabbage aphid. Disadvantageous influence of these extracts on aphids' survival was observed and this effect depended on extract's concentration.

Key words: glucosinolates, mustard, biological activity, aphids

I. INTRODUCTION

Glucosinolates, secondary plant metabolites characteristic for *Cruciferae* plants, such as rape (*Brassica napus* L.) and mustard (*Sinapis alba* L.) have been the object of our investigation for few years (Krzymańska et al. 1997; Waligóra 1997).

Lately, we have concentrated on glucosinolates occurring in mustard (*Sinapis alba*) conducting analytical and biological investigation of these compounds, to estimate their content in plant, properties and biological activity.

Mustard (*Sinapis alba* L.) is in Poland the plant of considerable economic importance for the sake of broad use. Even though mustard seeds are rich in protein of very profitable content of aminoacids and mineral salts, the presence of glucosinolates makes using of the mustard mash as a fodder impossible.

Antifeedant properties of glucosinolates and their biological activity in relation to different organisms was stated by many researchers (Mithen 1992; Drozdowska 1994; Giamoustaris and Mithen 1995; Oleszek 1995) and it had induced the interest in these compounds as a potential factor influencing the dynamic of pest population development (Doughty et al. 1991; Mithen 1992) or lower susceptibility of plant to pests, and also as a source of natural plant protection means.

Carrying on the research work on such type of compounds seems to be very profitable and effective from plant protection point of view, because the problem of seeking for non-chemical methods of protecting plants against pests is still topical.

II. METHODS AND MATERIALS

Designation of the content and composition of glucosinolates occurring in green parts of mustard was made. Four mustard varieties: Salvo, Metex, Ascot and Nakielska were taken into consideration. The analyses were made at different stages of plant development, to compare the course of metabolism of these compounds in investigated varieties.

The level of glucosinolates was analysed by HPLC (high performance liquid chromatography) method in four plant development stages: cotyledons, leaves, bud forming and inflorescences, using identical parameters as in previous years (Krzymańska et al. 1997). Three parallel samples were taken to the analyse in two replications. Final results are mean of 6 chromatographic designations.

In biological tests, the correlation between the appearance of glucosinolates in green parts of mustard and its susceptibility to aphids' infestation was investigated. The development of cabbage aphid (*Brevicoryne brassicae* L.) on four investigated varieties of mustard was observed. The rearing of insects was conducted in insulators, where on mustard plants (top part with flowers) 20 adult aphids were put on (for each replication) and the number of insects were recorded after 6 and 12 days of rearing. Experiments were made in 5 replications.

In tests concerning the biological activity of mustard glucosinolates – water and alcohol extracts from mustard leaves of Nakielska variety were used. The extracts were prepared by treating 10 g of leaves of mustard with 100 ml of boiling water (or boiling 70% ethanol), and after that shaking for two hours. The activity of these extracts was tested by rearing cabbage aphids on an artificial agar diet (control diet) supplied with tested extracts, in three different concentrations: 0.1%, 0.5% and 1 %. The diet was put into glass rearing vials – between two stretched membranes of Parafilm. For each combination ten replications were made, putting 5 aphids into each vial. Observations were made for 6 days.

III. RESULTS AND DISCUSSION

Results obtained in previously made designations indicated that the composition of glucosinolates in green parts of rape was different from their composition in seeds, so that was the reason why we took up the investigation concerning mustard. The aim of it was to establish the content and the composition of glucosinolates in green parts of mustard, which are the object of pests' infestation.

It is well known that in mustard seeds sinalbin makes up practically 100% of glucosinolates. Designating glucosinolates from green parts of mustard we had stated that in green parts of this plant also glucotropeolin, small amounts of glucobrassicanapine, and even traces of glucobrassicine appeared. The content of glucosinolates in different parts of plants belonging to particular varieties of mustard shows Table 1.

Analysing obtained results we have stated that in all mustard varieties sinalbin was the dominating component of glucosinolates in green parts of plant. The content of this compound rises along with plant's growing up and it differs significantly in particular plant organs. The smallest amount of sinalbin content (about 5 μM/g) was stated in cotyledons and the

Table 1

The content of glucosinolates in green parts of 4 mustard varieties – at different stages of vegetation – in μM/g

Variety	Glucosinolates	Cotyledons	Leaves	Flower buds	Inflorescences
Salvo	Sinalbin	4.57	11.78	15.31	37.26
	Glukonapin	–	0.01	–	–
	Glukobrassicanapin	0.01	0.03	0.04	0.09
	Glukotropeolin	0.01	0.15	3.75	8.56
	Glukobrassicyn	–	0.02	0.04	0.39
	Neoglukobrassicyn	–	–	0.02	0.08
	Unidentified	–	–	0.03	0.71
	Total	4.59	11.99	19.19	47.09
Metex	Sinalbin	4.55	15.22	12.03	42.19
	Glukonapin	–	–	–	śl.
	Glukobrassicanapin	0.02	0.03	0.03	0.08
	Glukotropeolin	tr.	0.13	4.27	17.68
	Glukobrassicyn	tr.	0.02	0.04	0.36
	Neoglukobrassicyn	–	–	0.02	0.10
	Unidentified	–	–	0.02	0.42
	Total	4.57	15.40	16.41	60.83
Nakielska	Sinalbin	5.14	14.83	12.92	43.12
	Glukonapin	–	–	–	0.04
	Glukobrassicanapin	0.01	0.04	0.03	0.10
	Glukotropeolin	0.02	0.29	4.33	8.87
	Glukobrassicyn	tr.	0.02	0.03	0.30
	Neoglukobrassicyn	–	–	0.02	0.09
	Unidentified	–	–	0.04	0.74
	Total	5.17	15.18	17.37	53.26
Ascot	Sinalbin	5.65	14.25	14.35	40.96
	Glukonapin	tr.	–	–	tr.
	Glukobrassicanapin	0.01	0.03	0.03	0.08
	Glukotropeolin	–	0.10	6.04	11.86
	Glukobrassicyn	tr.	0.01	0.04	0.35
	Neoglukobrassicyn	–	–	0.04	0.09
	Unidentified	–	–	0.06	0.81
	Total	5.66	14.39	20.56	54.15

higher in inflorescences (about 40 μM/g). In leaves the content of sinalbin appeared at the level of 12-15 μM/g. All these contents are similar for all investigated varieties.

Also, in all cases the appearance of glucotropeolin was stated, but in early stage of plant development the amount of this compound is very small, however the participation of it in total content of glucosinolates rises in flower buds and it is considerable in inflorescences.

Results showed in Table 1 indicate clearly that the participation of sinalbin in total glucosinolates content of cotyledons is practically 100% – it means it is the same as in seeds. In leaves, however, in spite of distinct absolute content of sinalbin its participation in

Table 2

Number of aphids on 4 mustard varieties (at flowering stage) after 6 and 12 days of rearing -mean from 5 replications

Variety	Initial number of aphids	Number of aphids after 6 days	Number of aphids after 12 days	Total amount of glucosinolates in μM/g
Salvo	20	112	196	37.3
Ascot	20	91	152	41.0
Metex	20	69	134	42.2
Nakielska	20	72	141	43.1

total amount of glucosinolates decreases a little and a very small amounts of glucotropeoline appears. Analyses of flower buds and inflorescences in which the level of total glucosinolates content is high, showed that sinalbin makes 70-80% of it, and glucotropeolin makes adequately 20-30%. It suggests, that the synthesis of glucosinolates in green parts of mustard plant goes independently from the synthesis of these compounds in seeds, where almost exclusively sinalbin is accumulated.

In biological experiments concerning the development of cabbage aphid (*Brevicoryne brassicae* L.) on different mustard varieties – top, flowering parts of plants were used. The content of glucosinolates in plants of this stage is rather high. Results obtained are shown in Table 2.

The highest number of aphids was stated, after 6 days as well as after 12 days of rearing, on Salvo variety, in which at the same time the level of glucosinolates was the lowest comparing to other varieties. On other varieties the number of insects was lower, and the level of glucosinolates content was higher. It suggests that higher level of glucosinolates in plant could cause some limitation of the development of aphids' population.

The results of experiments concerning biological activity of water and alcohol extracts from mustard leaves in relation to aphids' survival are presented in Table 3.

The best development of aphids was observed on control diet, although their development was not intensive. However, in all combinations with the addition of extracts, their disadvantageous influence on aphids' survival was observed. It depended on extract concentration. In experiments where water extract in concentrations of 0.5% and 1% were used – aphids practically did not survive 3 days. Only in the case of using the lowest concentration (e.g. 0.1%) of water extract insects lived for 6 days, and the number of individuals was

Table 3

Development of cabbage aphids (*Brevicoryne brassicae* L.) on the diet with addition of extracts from mustard leaves – mean from 5 replications

Day of rearing	Control	Water extract			Alcohol extract		
		0.1%	0.5%	1.0%	0.1%	0.5%	1.0%
1	5	5	5	5	5	5	5
2	12	14	7	5	5	2	1
3	12	16	3	2	3	1	–
4	10	12	1	–	2	–	–
5	9	12	–	–	1	–	–
6	9	11	–	–	–	–	–

even a little bit higher than in control combination. It would confirm the thesis, that there is a level of glucosinolates optimal for aphids development.

Stronger action in limiting pest's development showed the alcohol extract, which added to the diet caused total mortality of insects in short time, even in the lowest concentration. The reason of that was probably much higher glucosinolates content in this extract when comparing to water extract.

IV. LITERATURE

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GLUKOZYNOLANY WYSTĘPUJĄCE W CZĘŚCIACH ZIELONYCH GORCZYCY (*SINAPIS ALBA* L.) I ICH AKTYWNOŚĆ BIOLOGICZNA

STRESZCZENIE

Glukozynolany należą do tak zwanych wtórnych związków roślinnych, charakterystycznych dla roślin krzyżowych. Analizowano zawartość i skład tych związków w częściach zielonych gorczycy (*Sinapis alba* L), w czterech fazach rozwoju rośliny. Do badań wzięto 4 odmiany gorczycy: Salvo, Metex, Ascot i Nakielska. Stwierdzono, że w częściach zielonych gorczycy oprócz sinalbiny, która jest dominującym składnikiem glukozynolanów, występuje także glukotropeolina i niewielkie ilości glucobrassicanapiny. Zawartość tych związków zmienia się w czasie wegetacji i jest zróżnicowana w poszczególnych organach rośliny.

W doświadczeniach dotyczących biologicznej aktywności glukozynolanów gorczycowych testowano korelację pomiędzy zawartością tych związków w roślinie a jej podatnością na mszycę (*Brevicoryne brassicae* L.). Stwierdzono, że wyższy poziom zawartości glukozynolanów w roślinie powoduje pewne ograniczenie rozwoju szkodnika.

Testowano także aktywność biologiczną dwóch ekstraktów (wodnego i alkoholowego) z liści gorczycy w stosunku do mszycy kapuścianej. Obserwowano niekorzystny wpływ tych ekstraktów na przeżywalność mszyc i efekt ten zależny był od ich stężenia.