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PREPARATIONS OF ALKALOID-RICH LUPIN IN PLANT PROTECTION: AN EFFECT OF THE PREPARATIONS ON FEEDING AND DEVELOPMENT OF *PIERIS BRASSICAE* L. AND *PIERIS RAPAE* L.

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Abstract: A method for obtaining of alkaloid preparations (A_1 fraction and pure alkaloid perchlorates of A_5 one) from bitter lupin (*Lupinus albus* L. cv. Bac) extract and evaluation of their usefulness for plant protection are described. The activity of the extract as well as its fraction A_1 and two major alkaloids of A_5 one was tested on pests such as large cabbage white (*Pieris brassicae* L.) and small cabbage white (*Pieris rapae* L.) fed with leaves of cabbage treated with these preparations. Fractionation of the extract to obtain A_1 fraction and alkaloids perchlorates of A_5 one has been sufficient to eliminate impurities that may potentially cause undesirable by–effects in biological tests. Significant changes in various stages of development of insects were observed. Generally, fraction A_1 of lupin extract caused decrease in mass of fed feed as well as weight of objects observed. Furthermore, numerous visible damages of fed larvae and pupae that caused their inability to generate normal imagines (butterflies) were observed. Perchlorates of lupanine and 13–hydroxylupanine derived from lupin extract A_5 fraction have not shown that effect, particularly on *P. brassicae*. Above supports an evidence that only natural bioconjugates of active substance may perform strong biological activity.
In spite of similarity of tested insect species considerable differences between them in reaction to preparations were noticed.

Key words: white lupin, alkaloids, alkaloid bioconjugates, plant protection, large cabbage white, small cabbage white

INTRODUCTION

Pesticides, widely used in agricultural practice against agrophages are not neutral for human health due to traces thereof presented in food produced from treated plants as well as pollution of environment elements. Moreover, chemical, synthetic zoocides, the most harmful and dangerous for human pesticide group, have not eliminated any insect species from agrocenose ultimately.

Plants and insects have had simultaneous evolution what resulted in the creation of strong interactions between them. So, presently genetically dependent is choice of feed by various species of insects as well as are plant defense mechanisms against these pests.

Insects avoid plants containing defensive compounds however, some of them break the chemical barrier. Moreover, due to the formation of the specific mechanisms for detoxication by pests (Wink 1988; Boczek 1992; Nawrot 1998) these derivatives can even play a role of attractants for them. Fundamental meaning for recognition of feed by insects has had attractants and stimulants that were derived from allomones (Nawrot 1984). So, a lot of substances produced by plants have an influence on pest behavior.

Plant species have formed own defensive mechanisms against pests. Mainly they produce secondary metabolites, low toxic (tannins or enzyme inhibitors) and of high toxicity ones, e.g. alkaloids. They belong to self-defensive substances (Nawrot 1998) however, except that function may play, in interaction with biogenic polyamines and metal cations, significant role in transcription, translation and replication (Twardowski et al. 1982; Korcz et al. 1986).

Bitter lupin species are characterized by high content of quinolizidine alkaloids, bi-, tri- and tetracyclic ones. Total content of alkaloids in bitter species ranges from 0.2% to 4.0% of dry weight and in sweet ones from 0.05 to 0.2%. Set of alkaloids in lupin is characteristic for defined cultivars and partially used as a taxonomic criterion (Nowacki and Dunn 1964; Nowacki and Walker 1977). Various cultivars of the same species contain similar set of alkaloids but very often differ significantly in percentage.

The main aim of this study was to obtain the preparations from bitter white lupin (*Lupinus albus* L.) cv. Bac for their further use as potential products in plant protection. We work on the problem of complex utilization of lupin plant in wide agricultural and industrial practice since 1988. One of the by–product of lupin processing is an extract obtained during seeds debittering. The extract has contained saccharose, oligosaccharides (mainly raffinose family oligosaccharides, RFOs), organic acids, free aminoacids, peptides, mineral compounds and quinolizidine alkaloids. The last group of compounds called "a chemical weapon" of lupin plant (Wink 1984a; 1984b; 1988; Gulewicz 1988) was of interest of this work due to the possibility of their use as potential and ecological preparations in plant protection.

MATERIALS AND METHODS

Plant material

White lupin (*Lupinus albus* L.) cv. Bac seeds were a gift from Experimental Station at Przebędowo near Poznań, Poland.

Chemicals

25% Aqueous ammonia, chloroform, n-heksane, perchloric acid were purchased from POCh Gliwice, Poland. Methylene chloride and potassium hydroxide were from Odczynniki Chemicals, Lublin, Poland. Petroleum ether (Reactivul Bucuresti, Romania) and ethyl acetate (Chempur, Piekary Śląskie, Poland) were used. Separations were performed using Silica gel 60 plates (Merck, Germany) and Dowex 50 W × 8 cation exchange resin (Serva, Germany). All chemicals were of the analytical grade purity.

Lupin extract

Extract was generally obtained according to Gulewicz (1988; 1991), with slight modifications.

Seeds of lupin were kept to swell in distilled water for 8–12 hrs at 4°C. Then they were extracted twice with 48% ethanol (600 mL per 100 g of seeds). Supernatant was taken off and evaporated to 10% of starting volume (ca. 30% of dry weight).

Fractionation of lupin extract

Fractionation was performed according to the scheme given in figure 1. The extract (200 mL) was diluted with distilled water (1:1 v/v) and extracted twice with 400 mL of n-hexane. Hexane phase, called A_0 was separated. Water one was then extracted twice with 400 mL of methylene chloride (fraction A_1) followed by double extraction with 400 mL of ethyl acetate (fraction A_2).

Fractions A_0, A_1, and A_2 were evaporated to dryness. Water phase (ca. 50 mL) was separated by cation exchange chromatography (column of Dowex 50W × 8, 10 × 8 cm). Column was eluted with distilled water until an eluent of pH=5.5 resulted. An eluent contained A_3 and A_4 fractions separable by extraction with ethyl acetate. Column was then washed with 7*N* aqueous ammonia and eluate so obtained extracted with chloroform to obtain ammonia fraction A_6 and chloroform one A_5 (Stobiecki et al. 1993).

Isolation and purification of lupanine and 13-hydroxylupanine from fraction A_5

Fraction A_5 was dissolved in 20 mL of methanol and pH adjusted to 2.5 using perchloric acid. Crystallization was performed at 4°C for 1 hr giving pure crystals of lupanine perchlorate. Second crystallization yielded the mixture of salt of both alkaloids. Two grams of the mixture of perchlorates of lupanine and 13-hydroxylupanine were dissolved in water and alkalized with potassium hydroxide to pH=14. Sample was then separated using alkaline diatomite (kieselguhr) column that was eluted with petroleum ether (until the negative reaction with Drägendorf solution) followed by chloroform. Petroleum ether fraction contained pure lupanine and chloroform one 13-hydroxylupanine. Free alkaloids were then transformed to their perchlorate salts by acidification to pH=2.5 with perchloric acid.

Analysis of fractions A_1 and A_5

Analysis was performed using a Gas Chromatography – Mass Spectrometry (GC–MS) method by means of a Hewlett – Packard series II 5890 gas chromatograph equipped with a Hewlett – Packard 5971A Mass Selective Detector with electron ionization. Experiment was done using following temperature gradient: 2 min. at 180°C, followed by 5°C/min. to 300°C and 10 min. at 300°C. Temperature of the injector and detector was 290°C and 300°C, respectively.

Pests

Eggs of wild large cabbage white (*Pieris brassicae* L.) and small cabbage white (*Pieris rapae* L.) butterflies were collected from the fields located in the western region of Poland (Lądek District) where any plant protection products had not been used before. After hatching, larvae were fed with untreated cabbage leaves for one week. Larvae in L_2 – L_4 development stage were employed in all experiments.

Preparations for plant protection

Lupin seeds extract and its A_1 fraction as well as perchlorates of lupanine and 13-hydroxylupanine solutions obtained from A_5 fraction as potential insecticides or deterrents were applied.

To the solutions of preparations adjuvant Cittowet (BASF, Germany) was added, where 100% alkylarylpolyglycol ether is an active substance.

Greenhouse experiments

There were two types of experiments performed in greenhouse conditions. Basic one was done in six combinations and three replications, separately for *P. brassicae* and *P. rapae*. Leaves of cabbage were sprayed with 5% solution of lupin fraction or extract until run off. Five larvae, namely 3 in L_2 or L_3 stage and 2 in L_4 one were placed on the control and treated leaves and then located in round glass containers (ϕ = 12.5 cm) covered with sheet of gauze to avoid insects escape. Feed was supplemented during the experiment when necessary. Observations lasted about two weeks. In the beginning of the experiment and then every 48 hr leaves and larvae were weighted.

Transformation of larva into pupa of insects fed with cabbage plant growing in flower-pots were studied in additional experiment. Similarly to the basic one there were also six combinations in three replications, where one plant per pot constituted one replication. Plants were sprayed with 5% preparation and 5 larvae of large cabbage white and 5 ones of small cabbage white were placed on one plant. Pots were then located in the isolators. This experiment served studies on behavior of insects under conditions similar to the natural.

Combinations used in both types of experiment		Abbreviation
1)	control 1 – water	C
2)	control 2 – water + adjuvant	CAd
3)	lupin extract	ExL
4)	A_1 fraction of lupin extract	A_1
5)	13-hydroxylupanine perchlorate solution	OHLa
6)	lupanine perchlorate solution	La

All solutions were of 5% concentration. In combinations 2–6 adjuvant was added in concentration suggested by the manufacturer.

Based on our observations quantity of fed feed and increase of the mass of larvae and pupae were calculated. All data derived from the basic experiment and then expressed in tables 2 and 4 in Results and Discussion were elaborated statistically using the Student t test at the significance level of $p<0.005$.

For calculation of the absolute deterrence factor (Kiełczewski et al. 1979), the following equation was applied:

$$ADF = \frac{C-T}{C+T} \times 100$$

where:

C – mass of fed feed in the control 1 (water)

T – mass of fed feed treated with preparation

RESULTS AND DISCUSSION

Alkaloid composition of fraction A_1 and A_5 obtained at gentle and drastic conditions, respectively (Fig. 1) was established by means of GC–MS analysis and is compared in table 1. Based on retention times and the mass spectra library of natural compounds content of alkaloids in A_1 and A_5 fraction was determined and structures of most important ones are presented in figure 2. Fraction A_5 obtained after drastic treatment (Fig. 1) differs in alkaloids content from A_1 one (Tab. 1). Total percentage of alkaloids in A_1 and A_5 equals 1.0 and 18.5 of dry weight (DW), respectively. Moreover, fraction A_5 does not contain alkaloid esters, but mainly free bases of lupanine (10.3% DW), 13-hydroxylupanine (4.8% DW), and angustifoline (2.8% DW), derived probably from the esters, i.e. 13-tigloyloxylupanine, 13-angeloyloxylupanine and 13-tigloyloxymultiflorine that hydrolyzed during alkaline extract processing. These differences resulted in significantly different effect on insects between A_1 fraction and preparations obtained from A_5 one.

Table 2 refers to the basic experiment (see Materials and Methods) and describes an influence of preparations used in each combination (column I) on feeding and development of *P. brassicae* (col-

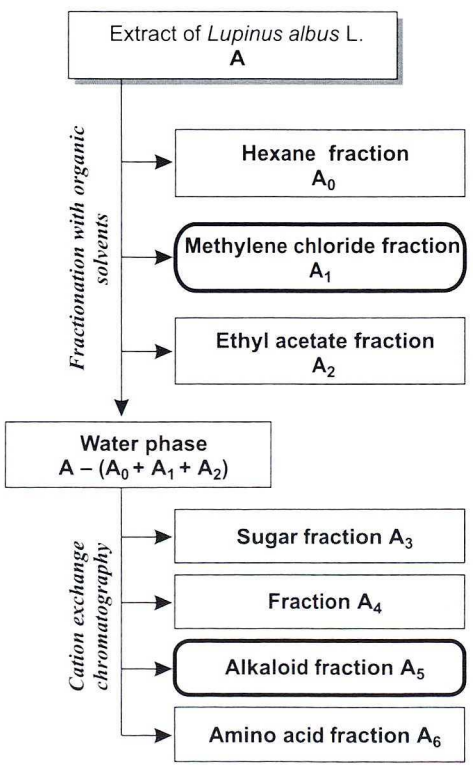

Fig. 1. Scheme of lupin extract fractionation

Table 1. Composition and percentage of alkaloids in fractions A_1 and A_5 from white lupin (*Lupinus albus* L.) cv. Bac seeds extract

Alkaloid	Fraction A_1 [%]	Fraction A_5 [%]
Sparteine	0.0140 ± 0.0000*	0.0572 ± 0.0009
Unidentified	ND**	0.0025 ± 0.0006
Angustifoline isomer	ND	0.0509 ± 0.0003
11,12-seco-12,13-Didehydromultiflorine	0.0085 ± 0.0007	0.0188 ± 0.0044
Angustifloline	0.0017 ± 0.0000	2.7653 ± 0.0013
Isolupanine	0.0179 ± 0.0010	0.0309 ± 0.0009
5,6-Didehydroxylupanine	0.0064 ± 0.0008	0.2047 ± 0.0022
Lupanine	0.7204 ± 0.0351	10.3006 ± 0.1200
11,12-Didehydroxylupanine	0.0562 ± 0.0032	0.0400 ± 0.0006
Multiflorine	0.0073 ± 0.0008	0.1903 ± 0.0016
13-Hydroxylupanine	0.0040 ± 0.0000	4.7997 ± 0.0672
13-Angelolyloxylupanine	0.0747 ± 0.0026	ND
13-Tigloyloxylupanine	0.0900 ± 0.0128	ND
13-Tigloyloxymultiflorine	0.0150 ± 0.0010	ND
Total	1.0161 ± 0.0262	18.4609 ± 0.0474

* – mean value ± SD (standard deviation)
** – not detected

umns II–VII). As shown in column II of the table, larvae of large cabbage white (*P. brassicae*) ate most readily leaves sprayed with solution of lupin seeds extract (ExL). The lowest level of feeding was observed in the combination pretreated with the solution of A_1 fraction (A_1). For both control combinations and those treated with lupin extract and solution of 13–hydroxylupanine salt (OHLa) level of eaten feed exceeded 20 g. Also difference in feed used between both controls 1 and 2 (C and CAd, respectively) was noticed.

Column III of the table 2 presents amounts of fed feed that was necessary for increase of mass of the larvae body in 1 mg. Quantities used for the 1 mg increase were highest in the cases of ExL, OHLa and A_1. The lowest level was observed for both, C and CAd combinations and the difference equaled 0.5 mg. Amounts of feed necessary for 1 mg increase in La sample was on the similar level however, higher than in both controls.

Changes in weight of large cabbage white larvae during experiment are presented in column IV. Taking to the consideration an initial and final weight it seems to be clear that the smallest is the difference in the case of A_1. In the combination pretreated with 13–hydroxylupanine perchlorate solution difference was on the similar level but significantly higher than in the case of A_1 combination. The largest differences in the control combinations were observed however, in the case of CAd one the difference was smaller. Differences in weight larger than 0.230 g were observed for lupin extract and lupanine perchlorate solution.

In column V of table 2, weight of pupae of *P. brassicae* at the end of the experiment is presented. It was lower than 0.200 g only in the combination A_1. In other cases weights were higher than 0.250 g and for ExL equaled even 0.324 g.

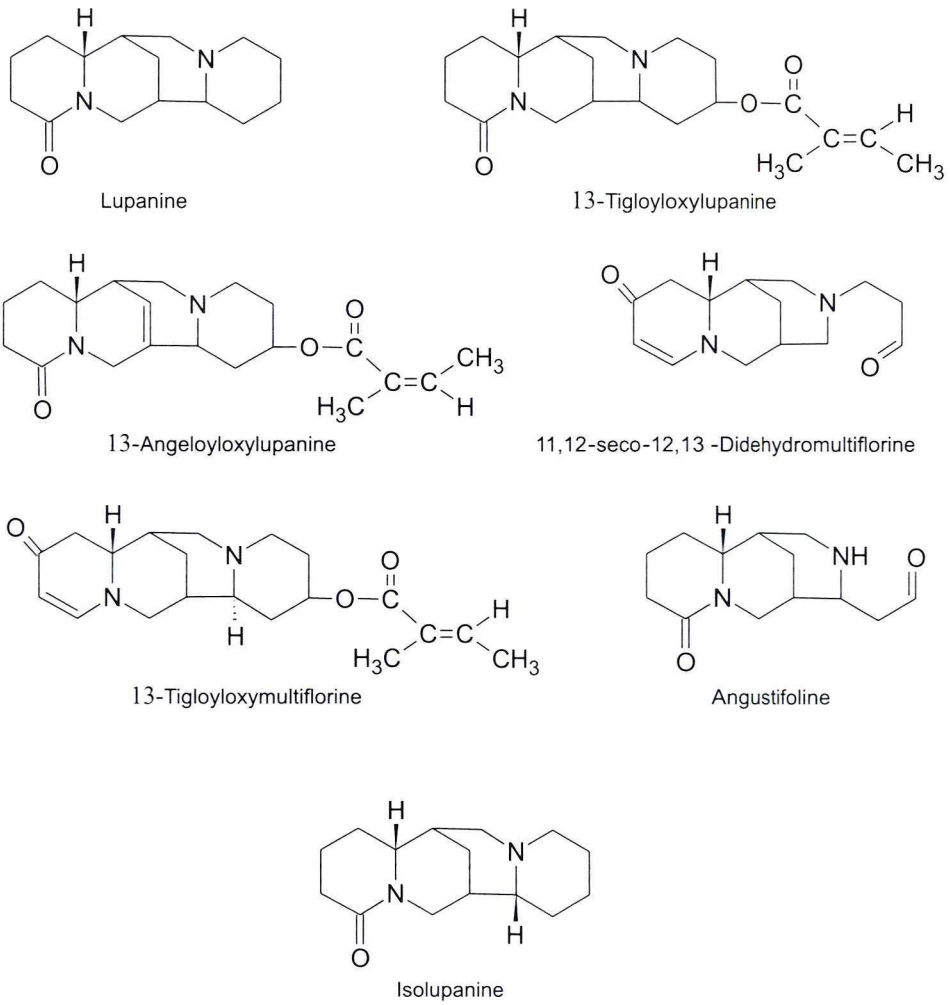

Fig. 2. Structures of most important alkaloids identified in A_1 fraction of white lupin cv. Bac extract

As we can see in column VI of the table the percentage of larvae transformed into pupae in ExL and OHLa combinations was the highest (93.3%). Slightly lower one (86.7%) was observed in La sample. The lowest transformation (40%) was noticed for A_1.

An absolute deterrence factor, *ADF* for *P. brasscae* was calculated using equation described in Materials and Methods. Values of the factor for each combination are presented in column VII. The highest one was noticed for A_1, 22.819. Contrary to others, combination using lupin extract (ExL) has had a negative value of *ADF* namely, –9.809.

Table 3 illustrates an effect of the preparation used in additional experiment (see Materials and Methods) on rate of transformation of larvae of large cabbage white

Table 2. Characteristics of the effect of preparations used in each particular combination on feeding and development of *Pieris brassicae* L.

Combination (Preparation)	Mass of used feed* [g]	Amount of feed used per 1 mg increase of larva mass* [mg]	Difference between starting and final weight of larvae* [g]	Weight of pupae in the end of experiment* [g]	Percentage of larvae transformed into pupae	Absolute deterrence factor *ADF*
I	II	III	IV	V	VI	VII
C	24.531 ± 0.542 A	17.046 ± 0.582 A	0.343 ± 0.043 A	0.274 ± 0.026 A	93.3 ± 11.5 A	
CAd	21.233 ± 0.208 B	17.586 ± 0.360 A	0.290 ± 0.018 B	0.296 ± 0.041 AB	93.3 ± 11.5 A	7.207
ExL	29.785 ± 1.122 C	27.341 ± 0.629 B	0.269 ± 0.017 B	0.324 ± 0.025 B	93.3 ± 11.5 A	–9.809
A_1	15.421 ± 0.533 D	27.013 ± 0.244 B	0.128 ± 0.004 C	0.203 ± 0.021 C	40.0 ± 20.0 B	22.819
OHLa	20.675 ± 1.577 B	27.605 ± 0.930 B	0.189 ± 0.024 D	0.269 ± 0.009 A	93.3 ± 11.5 A	8.529
La	20.126 ± 0.262 B	20.156 ± 0.983 C	0.234 ± 0.041 B	0.264 ± 0.005 A	86.7 ± 23.1 A	9.865

* Mean value ± SD (Standard Deviation). Capital letters A – D following entries indicate a statistical significance at $p<0.005$

Table 4. Characteristics of the effect of preparations used in each particular combination on feeding and development of *Pieris rapae* L.

Combination (Preparation)	Mass of used feed*[g]	Amount of feed used per 1 mg increase of larva mass* [mg]	Difference between starting and final weight of larvae* [g]	Weight of pupae in the end of experiment* [g]	Percentage of larvae transformed into pupae	Absolute deterrence factor *ADF*
I	II	III	IV	V	VI	VII
C	10.471 ± 0.415 A	16.009 ± 0.538 A	0.123 ± 0.016 A	0.144 ± 0.002 A	93.3 ±11.5 A	
CAd	9.017 ± 0.274 B	14.289 ± 0.447 B	0.142 ± 0.006 B	0.136 ± 0.001 AB	100.0 ± 0.0 A	7.462
ExL	9.650 ± 0.266 C	19.246 ± 1.070 C	0.141 ± 0.007 B	0.134 ± 0.001 B	93.3 ± 11.5 A	4.084
A_1	10.214 ± 0.220 A	22.091 ± 0.246 D	0.070 ± 0.006 C	0.123 ± 0.004 C	60.0 ± 20.0 B	1.247
OHLa	10.110 ± 0.364 A	15.435 ± 0.286 BE	0.148 ± 0.007 D	0.138 ± 0.007 AB	80.0 ± 20.0 C	1.759
La	8.907 ± 0.132 B	16.494 ± 0.333 E	0.095 ± 0.009 E	0.131 ± 0.008 AB	73.3 ± 20.0 C	8.073

* Mean value ± SD (Standard Deviation). Capital letters A – E following entries indicate a statistical significance at $p<0.005$

Table 3. Number of transformations of *Pieris brassicae* L. larvae into pupae every other day of the experiment

Combination	Day of experiment								
	Start*	2	4	6	8	10	12	14	End**
	Number of transformations								
C	5 5 5			3	2 1	3			5 4 5
CAd	5 5 5		1	5 1	1	1 3	1 1		5 4 5
ExL	5 5 5			3 1†† 1	2 1	1 2	3 1		5 4; (1††) 5
A_1	5 5 5			1 1		1; 1†† 1†	2		1; (1††) 1; 1† 3
OHLa	5 5 5			4 1 1		1 1 3	1	2	5 4 5
La	5 5 5			4 1 1	3	1	1	1 1	5 3 5

* – starting number of larvae
** – final number of pupae
1 – normal pupa
1† – incorrectly developed pupa
1†† – dead larva

into pupae and their mortality. One incorrectly developed pupa and one dead larva were observed for A_1 fraction. Lupin extract produced also one dead larva while other combinations showed insects correctly developed.

Table 4 refers again to the basic experiment and characterizes an influence of preparations used in each combination (column I) on feeding and development of *P. rapae* (columns II–VII).

In column II of table 4 quantities of fed feed by small cabbage white (*P. rapae*) are given. It seems to be clear that there were not significant differences between combinations studied. Difference between the highest (control 1, C) and the lowest (lupanine fraction, La) amount eaten by larvae equaled 1.564 g.

Column III presents an amount of feed needed for the 1 mg increase of *P. rapae* larva mass. The largest value is observed for A_1 combination (22.1 mg) and the smallest one noticed for control 2 with adjuvant (14.3 mg). Amounts for C, OHLa and La samples were similar and equaled 16 mg, 15.4 mg and 16.5 mg, respectively.

Results presenting changes in weight of small cabbage white larvae during the experiments are given in column IV of table 4. There were significant differences between each particular combination. The highest increase in weight was observed

for OHLa one, and slightly lower for ExL and CAd. The lowest values were observed for A_1 (0.070 g). Amount of eaten feed in La combination was also low as 0.095 g.

The lowest weight of pupae of small cabbage white was noticed in A_1 combination, 0.123 g (Column V, Tab. 4). Pupae of the highest weight were observed for control 1 combination, 0.144 g. Weight of pupae for the rest of combinations ranged between 0.131 g and 0.138 g.

The highest percentage of transformation of *P. rapae* larvae into pupae was observed for the control combination 2, CAd (100%) and C and ExL ones, 93.3 each. The lowest value was noticed for A_1 (60%). In the combinations OHLa and La the rate was lower than in both controls and ExL but higher than in the rest ones (ColumnVI, Tab. 4).

In column VII values of the absolute deterrence factor (*ADF*) for *P. rapae* are compared for each particular combination. The highest one was noted for La (8.073) and the lowest observed for A_1 (1.247).

Table 5 contains results concerning the number of transformations of the larva of small cabbage white into its pupae and their mortality in the additional experiment. Incorrectly developed pupae were observed for ExL (one individual) and

Table 5. Number of transformations of *Pieris rapae* L. larvae into pupae every other day of the experiment

Combination	Day of experiment								
	Start*	2	4	6	8	10	12	14	End**
	Number of transformations								
C	5 5 5			3 1; 1†† 2	2 1	 1	 2	1 1	5 4; (1††) 5
CAd	5 5 5			 3	2 1	3 3	 1	 2	5 5 5
ExL	5 5 5	1††		2 1	2; 1† 1	 1	 1		 4; 1† 4; (1††)
A_1	5 5 5	 1††			 1; 1†† 2	2 3	 1†† 1		3; (1††) 2; (2††) 4
OHLa	5 5 5	 1			1 2	3 1; 1† 1	1† 2		4; 1† 3; 1† 3
La	5 5 5			 1; 1††	1 2	4 2	 1; 2††		5 3; (2††) 3; (2††)

* – starting number of larvae
** – final number of pupae (dead larvae)
1 – normal pupa
1† – incorrectly developed pupa
1†† – dead larva

OHLa (two ones). For C, ExL, and OHLa one in each combination dead larva was noticed. Two of them were found in combination La and three in the A_1.

Spraying of the cabbage leaves with lupin extract in basic experiment had a positive effect on feeding of large cabbage white larvae that resulted in the negative value of the absolute deterrence factor *ADF*. In the case of small cabbage white so strong effect has not been observed. Stimulating effect of lupin extract on insect feeding could be explained by a high content of sugars (saccharose and raffinose family oligosaccharides, RFOs) that reached up to 50% of dry mass while alkaloids constitute about 10% of the pool (Gulewicz 1988). Sugars are probably a valuable energetic material for *P. brassicae*, especially that preparations of sugarless fraction A_1 does perform an inverse activity. It is not in agreement with observations of other researchers (Wyrostkiewicz et al. 1996; Wyrostkiewicz and Wawrzyniak 1997), but they used extracts of different lupin cultivars. Fraction A_1 showed the highest negative effect on feeding and development of large cabbage white. This preparation from white lupin seeds contains quinolizidine alkaloids in bioconjugate form that appears in the nature namely, esters, salts etc. Large difference in biological activity between A_1 fraction and pure alkaloid perchlorates (La fraction and OHLa one, both derived from A_5 fraction) supports an evidence that only natural conjugates of active substance have an ability to function as "a chemical weapon" of lupin and potential insecticides or repellents. It is in an agreement with observations of others (Wink 1994; Muzquiz et al. 1997; Wyrostkiewicz and Wawrzyniak 1997). In the case of small cabbage white an effect of A_1 on feeding was not so strong but this preparation affected increase of the mass of larvae as well as the rate of their transformation into pupae that was also confirmed by others (Wyrostkiewicz et al. 1996; Wyrostkiewicz and Wawrzyniak 1997).

Simultaneous observations of the behavior of larvae in additional experiment fed with cabbage growing in isolators were in full agreement with basic one. During the experiment intensive feeding of both species on controls, lupin extract, 13-hydroxylupanine and lupanine combinations was observed contrary to A_1 fraction where larvae looked for feed and some week or dead individuals were found. Toxic properties of quinolizidine alkaloids are known. Nevertheless, behavior of insects, especially *P. rapae* in this experiment showed that mortality of larvae and abnormality of pupae could be a result of poisoning as well as starvation caused by deterrent or repellent influence of the preparations.

Two-week experiment period allowed us for observation of an effect of preparation used on transformation of the larvae into pupae of both species. It was interesting to find incorrectly developed pupae in A_1 combination and for small cabbage white also in ExL and OHLa ones. In the case of pupae incorrectly developed, transformation was not terminated while all correctly developed ones were transformed into normal imagines.

CONCLUSIONS

Generally, we can conclude that in the case of large cabbage white fraction A_1 of lupin extract was the most efficient preparation that resulted in low level of feeding, a high value of an absolute deterrence factor, low weight of pupae and low rate

of transformation of larvae into pupae. Non processed, raw lupin extract stimulated feeding of large cabbage white larvae that resulted in the negative value of the deterrence factor (–9.809), a high weight of pupae and rate of transformation.

Increase in weight of small cabbage white larvae were the lowest in the case of using of A_1 fraction however, it was not correlated with the amount of eaten feed ($ADF = 1.247$).

Feeding with cabbage sprayed with A_1 fraction and extract as well as 13-hydroxylupanine perchlorate caused damages of pupae of small cabbage white.

There is no significant influence of alkaloid perchlorates used on feeding and development of tested species. Above supports an evidence that active substance, but only in form acceptable by pest is able to perform strong biological activity analogously to dependence of drug efficacy on its formula.

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POLISH SUMMARY

PREPARATY Z WYSOKOALKALOIDOWEGO ŁUBINU W OCHRONIE ROŚLIN: WPŁYW TYCH PREPARATÓW NA ŻEROWANIE I ROZWÓJ *PIERIS BRASSICAE* L. I *PIERIS RAPAE* L.

Opisano metodę uzyskiwania preparatów alkaloidowych (frakcji A_1 i czystych nadchloranów alkaloidów frakcji A_5) z ekstraktu nasion łubinu gorzkiego (*Lupinus albus* L. odm. Bac) oraz oszacowano ich przydatność, jako środków ochrony roślin. Aktywność ekstraktu a także jego frakcji A_1 oraz dwóch głównych alkaloidów frakcji A_5 była testowana na takich szkodnikach, jak bielinek kapustnik (*Pieris brassicae* L.) i bielinek rzepnik (*Pieris rapae* L.) karmionych liśćmi kapusty traktowanymi przedtem tymi preparatami. Frakcjonowanie ekstraktu w celu otrzymania frakcji A_1 i nadchloranów alkaloidów frakcji A_5 wystarczało do wyeliminowania zanieczyszczeń, które mogłyby potencjalnie powodować niepożądane efekty uboczne w testach biologicznych. Zaobserwowano znaczne zmiany w różnych stadiach rozwoju owadów. Ogólnie, frakcja A_1 powodowała zmniejszenie masy zjedzonego pokarmu oraz wagi obserwowanych obiektów. Ponadto, notowano liczne, widoczne uszkodzenia larw i poczwarek, co powodowało ich niezdolność do przekształcenia się w normalnego motyla. Nadchlorany lupaniny i 13-hydroksylupaniny otrzymane z łubinowej frakcji A_5 nie powodowały takich skutków, zwłaszcza wobec *P. brassicae*. Dowodzi to, że tylko naturalne biokoniugaty aktywnych substancji mogą wykazywać silną aktywność biologiczną.

Pomimo podobieństwa testowanych gatunków odnotowano znaczne różnice pomiędzy nimi pod względem reakcji na preparaty.