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EFFECT OF ALFALFA (*Medicago sativa* L.) SAPONINS ON DEVELOPMENT AND FERTILITY OF GRAIN WEEVIL (*Sitophilus granarius* L.)

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Abstract. The direct and subsequent effects of saponins obtained from aboveground parts of alfalfa (*Medicago sativa* L.) on development and fertility of grain weevil (*Sitophilus granarius* L.) were investigated. The results have revealed that life cycle of grain weevil prolongs along with an increase in saponin concentration, whereas duration of the offspring generation emergency decreases. Also, a significant reduction in grain weevil fertility was observed. Strong subsequent effect of saponins on the offspring generation bred on untreated wheat was recorded. Prolongation of the life cycle and duration of the offspring generation emergency of grain weevil was noted in comparison with the results from tests of the direct effect. Moreover, the fertility index of the offspring generation was lower.

Key words: saponins, alfalfa, *Sitophilus granarius*

INTRODUCTION

There has been observed a tendency to replace chemical insecticides used in plant protection and protection of stored products against pests, with products that are natural compounds of plants. The application of natural compounds acting as insecticides or deterrents capable of inhibiting fertility and feeding of pests may significantly reduce the amount of the chemical products utilized. Plants either contain or produce this type of compounds. Both basic feeding substances necessary for normal metabolism of pests (amino acids, monosaccharides, lipids) and secondary plant metabolites (terpenoids, alkaloids, saponins, coumarins, glycosides, phenolic substances, thiocyanates) can negatively influence the pest biology [Beck 1965, Beek and Groot 1986].

The hypothesis assumes that natural saponins may be come a potential source of alternative substances for chemical plant protection and storage pests. The main goal of the investigations was to examine the direct and subsequent influence of saponins obtained from aboveground parts of alfalfa (*Medicago sativa*) on the development and fertility of grain weevil (*Sitophilus granarius* L.).

MATERIAL AND METHODS

S. granarius was covered by the studies. Weevil was bred in glass flasks filled with consumption wheat grains in laboratory conditions at 25°C and the relative humidity of 70-80%. Saponins were obtained from aboveground parts of alfalfa [Oleszek et al. 1992]. Wheat grains were first sprayed with water solutions of saponins or with water (10 ml/100 g) and next dried up at room temperature and placed into flasks. Two experiments were set up and each of them comprised 5 series, 10 replications in each. One glass flask was one replication. In the first experiment the saponins were added into wheat grains at the doses of 1000 ppm – series I, 100 ppm – series II, 10 ppm – series III, 1 ppm – series IV and wheat grains treated with distilled water (control) – series V.

Ten males and ten females 2-5 days of age were placed in flasks filled with 100g of wheat grains. Each flask was tightly covered with mill gauze. Twenty days after the establishment of the experiment, all beetles were removed. Next, after first individuals of the offspring generation emerged, the flasks were checked every other day, the new beetles were counted and removed. The checking and counting was performed as long as new individuals were emerging.

To study the sequent effect of the saponins, the second experiment was set up without the saponin supplement and wheat grains treated with distilled water. The beetles collected during the first experiment were used (2-5 days of age) in the second experiment. The beetles from series I (F1) became Ia, series (F2), from series II – IIa, from series III – IIIa, from series IV – Iva, and from control – Va.

Each series included 10 replications. The averaged results obtained in the control series of both experiments were presented as the control.

The fertility index was defined for each series. As in all the experimental series females constituted half of the whole population introduced, the fertility index was calculated based on the correlation of the number of individuals from the offspring generation to the number of females of the parental generation [Błażejewska 1969].

The results were described statistically by applying variance analysis of single orthogonal experiments. The object means were measured with the use of Tukey's test.

RESULTS AND DISCUSSION

The results indicated that the saponins tested showed biological activity against grain weevil. The saponin supplement affected a development of *S. granarius*. Observations on the direct effect of saponins revealed that life cycle of grain weevil prolongs along with an increase in saponin concentration and for saponins at the highest dose: 1000 ppm caused a significant prolongation as compared to the cycle observed in tests where lower saponins doses were given, and in the controlled test. However, the duration of the offspring generation emergency decreases (Table 1). The time is significantly shorter in the combination where the highest dose of saponins was added to the culture than in the other experimental combinations.

There was also noted a significant pest fertility decline. The fertility index for grain weevil was the highest in the control series and in the series with the lowest dose of saponins i.e. 1 ppm (Table 2). In the remaining series the value of the index decreased along with saponin concentration increases.

Table 1. Effect of saponins obtained from aboveground parts of *Medicago sativa* on grain weevil development
Tabela 1. Wpływ saponin wyizolowanych z części nadziemnych lucerny siewnej na rozwój wołka zbożowego

Experiment Doświadczenie	Series – Seria dose of saponins/ 100 g grain dawka saponin/ 100 g ziarna ppm	Occurrence of imago generation, days Czas wylęgu chrząszczy, dni			Generation development, days Czas rozwoju pokolenia, dni		
		min	max	medium średni	min	max	medium średni
1 – with saponins 1 – z dodatkiem saponin	I (1000)	20	33	26.3 d	31	39	36.4 a
	II (100)	25	37	31.2 c	28	38	32.3 ab
	III (10)	27	37	31.9 bc	28	31	29.5 b
	IV (1)	29	36	33.5 ab	28	30	28.6 b
	V (0)	32	36	34.4 a	28	30	28.4 b
	$LSD_{0.05} – NIR_{0,05}$			1.956			5.763
2 – without saponin supplement 2 – bez saponin	Ia (0)	32	41	37.2 b	43	50	44.8 a
	IIa (0)	32	41	36.0 bc	43	50	44.7 a
	IIIa (0)	30	41	35.2 c	43	43	43.0 a
	IVa (0)	34	41	39.5 a	43	43	43.0 a
	Va (0)	32	38	34.4 c	28	30	28.4 b
	$LSD_{0.05} – NIR_{0,05}$			1.723			3.354

The statistical analysis revealed significant differences between an average number of individuals of the offspring generation obtained from series with the highest saponin concentration (I and II) and the remaining series III, IV and V. There were no significant differences found between series IV (1 ppm) and the control.

There was observed a powerful subsequent effect of saponins on the F2 generation of grain weevil bred on wheat grains untreated with the compounds tested. The life cycle of the *S. granarius* and duration of the offspring generation emergency was recorded as compared to the results from the experiment on direct influence (Table 1). Moreover, the fertility index of the offspring generation was lower (Table 2).

The statistical analysis showed significant differences between an average number of individuals of the F2 generation obtained from control, the series with the highest saponin concentration and the remaining series.

There were no dead beetles found in both experiments (Table 2).

In the available literature no data concerning penetration of saponins through coccidiom coats was found. A decrease in F2 generation fertility is undoubtedly related to the contact and feeding of the imago generation F1 with grain treated with saponines.

Saponins of *M. sativa* show biologically active against insects and pathogenic microorganisms. They are a complicated mixture of triterpenoid glycosides. Depending on aglycone structure, glycosides of medicagenic acid, zanic acid, hederagenins, bayogenins and soyasapogenols A, B and E can be present. Commonly the biological activity of saponins is reported to be modified by aglycone structure. The saponins tested from aboveground parts of alfalfa are mixtures of glycosides of medicagenic acid (at a lower concentration than in roots) and glycosides of zanic acid and soyasapogenol [Oleszek et al. 1992]. Krzymańska and Waligóra [1983] have proved that in leaves of *M. sativa* resistant to pests, medicagenic acid is a dominating compound of saponins.

Table 2. Effect of saponins obtained from aboveground parts of *Medicago sativa* on grain weevil fertility
Tabela 2. Wpływ saponin wyizolowanych z części nadziemnych lucerny siewnej na płodność wołka zbożowego

Experiment Doświadczenie	Observations Obserwacje	Number of samples Liczba prób	Series – Seria dose of saponins/100 g grain – dawka saponin/100 g ziarna ppm					$LSD_{0.05}$ $NIR_{0,05}$
			I (1000)	II (100)	III (10)	IV (1)	V (0)	
1 – with saponins 1 – z dodatkiem saponin	Mean number of individuals from F1 generation Średnia liczba osobników pokolenia F1	10	2109	2827	3367	4430	4283	113.833
	Fertility index Wskaźnik płodności		21.09	28.27	33.67	44.30	42.83	
2 – without saponins supplement 2 – bez saponin	Mean number of individuals from F2 generation Średnia liczba osobników pokolenia F2	10	380	621	443	1713	4283	39.969
	Fertility index Wskaźnik płodności		3.80	6.21	4.43	17.13	42.83	

Alfalfa saponis were toxic to European meal worm *Tenebrio molitor* [Pracros 1982], grape berry moth – *Lobesia botrana* [Tava et al. 1992], European maize borer – *Ostrinia nubilalis* [Nozzolillo et al. 1997], cotton leafworm – *Spodoptera litoralis* [Adel et al. 2000], potato leafhopper – *Empoasca fabae*, hop aphids – *Phorodon humuli,* two-spotted spider mite – *Tetranychus urticae* [Puszkar et al. 1994], Colorado potato beetle – *Leptinotarsa decemlineata* [Waligóra and Krzymańska 1994].

The present results suggest that saponins also cause disorders in the development and fertility of grain weevil.

CONCLUSSIONS

1. Saponins separated from aboveground parts of alfalfa (*Medicago sativa* L.) affect the development and fertility of grain weevil (*Sitophilus granarius* L.)
2. As for the direct effect, the life cycle of the grain weevil prolongs along and duration of the offspring generation emergency decreases with an increase in saponin concentration, and fertility index decreases.
3. As for the subsequent effect, there are observed the prolongation of the life cycle and duration of the offspring generation of the grain weevil, as compared with the direct activity, and the fertility index also decreases.

REFERENCES

Adel M.M., Sehnal F., Jurzysta M., 2000. Effects of alfalfa saponins on the moth *Spodoptera litoralis*. J. Chem. Ecol. 26 (4), 1065-1078.

Beck S.D., 1965. Resistance of plants to insects. Ann. Rev. Ent. 10, 207-232.

Beek van T.A., de Groot A., 1986. Terpenoid antifeedants. Part. I, An overview of terpenoid antifeedants of natural origin. Rec. Trav. Chim. Pays-Bas 105, 513.

Błażejewska A., 1969. Płodność wołka zbożowego (*Calandra granaria* L.) hodowanego na ziarniakach pszenicy traktowanej chlorkiem chlorocholiny (CCC) [Fertility of grain weevil (*Calandra granaria* L.) bred on wheat grains treated with CCC]. Rocz. Nauk Roln. 95, 395-399 [in Polish].

Krzymańska J., Waligora D., 1983. Znaczenie saponin w odporności lucerny na mszycę grochową (*Acyrtosiphon pisum* H.) [Importance of saponins in alfalfa resistance to pea aphid (*Acyrtosiphon pisum* H.)]. Prace Nauk. IOR 24 (2), 153-160 [in Polish].

Nozzolillo C., Arnason J.T., Campos R., Donskoy N., Jurzysta M., 1997. Alfalfa leaf saponins and insect resistance. J. Chem. Ecol. 23, 995-1002.

Oleszek W., Jurzysta M., Górski P., 1992. Alfalfa saponins – the allelopathic agents. [In:] Frontiers of Allelochemical Research, Rizyi S.J.H., Rizyi V. (eds.), Chapman & Hall. London, 151-164.

Oleszek W., Jurzysta M., Płoszyński M., 1992. Zanhoc acid tridesmoside and other dominant saponins from alfalfa (*Medicago sativa* L.) aerial parts. J. Agric. Food Chem. 40, 191-196.

Pracros P., 1982. Intéret comparé de 1`éstimation biologique de la valeur nutritionnelle des sources protéiniques par des vértébres (rats et poulets) et par un insecte (*Tenebrio molitor* L.). C.R. Acad. Agric. Fr., 1279-1285.

Puszkar A., Jastrzębski M., Jurzysta M., Biały Z., 1994. Saponiny lucerny jako szansa w integrowanej ochronie chmielu [Alfalfa saponins as a chance in hop integral protection]. Mat. XXXIV Sesji Nauk. IOR 2, 255-259 [in Polish].

Tava A., Forti D., Odoardi M., 1992. Alfalfa saponins: Isolation, chemical characterization and biological activity against insects. Proceedings, X *Eucarpia medicago* spp., Group Meeting, Lodi, Italy, 283-288.

Waligóra D., Krzymańska J., 1994. Wpływ roślinnych związków wtórnych – glukozynolanów, alkaloidów i saponin na żerowanie stonki ziemniaczanej (*Leptinotarsa decemlineata* Say.) [Effect of plant secondary compounds: glucosinolates, alkaloids and saponins on Colorado beetle (*Leptinotarsa decemlineata* Say.) feeding]. Mat. XXXIV Sesji Nauk. IOR 2, 9-12 [in Polish].

WPŁYW SAPONIN Z LUCERNY SIEWNEJ (*Medicago sativa* L.) NA ROZWÓJ I PŁODNOŚĆ WOŁKA ZBOŻOWEGO (*Sitophilus granarius* L.)

Streszczenie. Badano bezpośredni i następczy wpływu saponin uzyskanych z części nadziemnych lucerny siewnej (*Medicago sativa*) na rozwój i płodność wołka zbożowego. Wykazano, że wraz ze wzrostem stężenia saponin cykl rozwojowy wołka zbożowego wydłuża się, natomiast skraca się czas trwania pojawu chrząszczy pokolenia potomnego. Obserwowano również istotne obniżenie płodności szkodnika. Zanotowano silne działanie następcze saponin na pokolenie potomne hodowane na pszenicy nie traktowanej. Stwierdzono wydłużenie cyklu rozwojowego wołka i czasu trwania pojawu pokolenia potomnego w porównaniu z notowanym w działaniu bezpośrednim. Uzyskano również niższy wskaźnik płodności pokolenia potomnego.

Słowa kluczowe: saponiny, lucerna siewna, wołek zbożowy

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