

## ACTIVITY OF THE SAPONIN EXTRACT FROM THE BARK OF *QUILLAJA SAPONARIA* MOLINA, AGAINST COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY)

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**Abstract:** The paper reports results of investigations concerning the influence of saponin extract of quillaja on the food choice and development of Colorado potato beetle. Choice tests with the use of potato leaf discs treated and untreated with tested saponins' extract were made. The influence of the presence of saponins on the food choice and intensity of insect feeding were observed. It was stated that saponins significantly restrain feeding and disturb development of this pest.

**Key words:** Colorado potato beetle, saponins, biological activity, potato protection

### INTRODUCTION

Colorado potato beetle (*Leptinotarsa decemlineata* Say) (CPB) is still one of the most severe pest. Every year this pest has caused a great reduction of potato yield, because it attacks the plant of great economic significance, widely cultivated and broadly utilized especially in Poland. Colorado potato beetle possesses a great ability of adaptation to new conditions and quickly acquires the resistance to applied insecticides (Cheeke 1971; Węgorzek et al. 1988; Węgorzek 1995; Pawińska and Węgorzek 1998; Noronha et al. 1999). Because of that, it seems to be necessary to look for a new, alternative and efficient methods of Colorado potato beetle control.

In this aspect very interesting are secondary plant metabolites-chemical compounds naturally produced by plants. This type of compounds often shows defined, mostly negative influence on pest behavior what may result in changes of some their life processes. One of such a group of secondary plant metabolites, which naturally occur in many plant species are saponins. Their chemical structure is rather complicated and as it was stated they often differ in their biological activity and physiological properties. It was found that saponins showed antibiotic or even toxic activity against many organisms (Gestetner et al. 1971; Horber 1972; Krzymańska and

Waligóra 1983; Nozzolillo et al. 1997; Waligóra 1998; Adel et al. 2000; Saniewska et al. 2001) and also allelopathic activity (Oleszek and Jurzysta 1987; Waller et al. 1995). There is a potential possibility of using these compounds in plant protection. Investigation on this group of metabolites is important because, if even such compounds can not be used directly in pest control, they can give suggestion for breeding new, more resistant varieties or inspiration in searching new synthetic insecticides.

For some years we have been working on saponins and their relation to Colorado potato beetle. Because saponins are rather complicated compounds and they differ in their chemical structure and biological activity it is important to inquire into saponins originated from different plants to compare their properties. To obtain as much as possible information concerning the influence of saponins on Colorado potato beetle feeding and behavior we had tested saponin extracts isolated from few different plants such as: *Medicago sativa* L., *Saponaria officinalis* L., *Convallaria maialis* L. and *Herniaria glabra* L. Obtained results are interesting and hopeful, so the next step in our investigation are saponins appearing in the extract from the bark of *Quillaja saponaria* Molina.

The object of the investigations was total saponin extract from the bark of *Quillaja saponaria* Molina, which is an evergreen tree belonging to *Rosaceae* family, growing wild in South America. The bark of this plant which is rich in saponins (about 20%) is a medicinal raw material (*cortex quillaiiae*). Powder and dust of quillaja bark cause strong irritation of man mucous membranes of nose and throat effecting in sneezing and coughing because of the presence of saponins in it (Bocheńska 1992).

## MATERIALS AND METHODS

Tested material was ready-made commercial product sold as a white-gray powder, which is used in therapeutics as expectorant in lungs disease and lasting bronchial inflammation. From such a powder we prepared 1% aqueous solution that in this form was used in all biological experiments concerning the influence of *Q. saponaria* extract on Colorado potato beetle food choice, feeding and development of this pest.

To determine the influence of tested extract on food choice by Colorado potato beetle choice and no choice tests were carried out. In choicetests insects could choose treated (with saponin extract) or untreated (control) food and in nochoice test only treated food was given to them. Experiments were conducted in laboratory conditions, at room temperature (20–25°C) and daylight. Tests were carried on in Petri dishes (9 cm in diameter) laid with moisten filter paper on which disks cut out from the potato leaves were placed. In case of choicetests four discs were put into each dish: two untreated discs (control) and two discs treated with tested extract. Instead in case of nochoice tests all four discs were treated with tested extract while control combination consisted of four untreated leaves. Saponin extract dissolved in water at the concentration of 1% was used in each case. Fresh cut leaf discs of defined surface area were dipped in saponin extract solution for a few seconds and then placed in dishes.

Both larvae and beetles of Colorado potato beetle were used in experiments. In each dish (one replication) two larvae of third-instar or two beetles were placed. After two hours of feeding insects were removed and residues of leaf discs were scanned. The area of remaining discs was measured using a specially elaborated computer program and intensity of insects' feeding was determined calculating the size of eaten area in each experimental combination. Tests were made in 5 series, each of them consisted of 5 replica-

tions (5 dishes) containing 4 discs therefore, for each experimental combination 100 discs were tested. Statistical analysis was made using WinSTAT program, version 2001.1

To define the influence of tested extract on the development of Colorado potato beetle young larvae ( $L_2$ ) of this insect were reared on potato leaves sprayed with this extract and their survival, feeding and weight gain was observed. Rearing of insects was conducted in spring-summer season (from the beginning of May to the end of July) in the insectarium.

Larvae of second-instar were weighed and placed on potato leaves. In control combinations leaves stayed untreated while in experimental combination leaves were sprayed with 1% solution of saponin extract from quillaja. Experiments were done in two series. Each series was set up in 4 replications – 25 larvae per replication. Observations were performed every third day. Survival, intensity of feeding and weight gain of larvae were noted.

## RESULTS AND DISCUSSION

In case of all choice and nochoice experiments the area of remaining discs was measured and the surface of standard leaf disc was established – as a mean area of all control discs (its surface amounted to 280.05 mm<sup>2</sup>). Then, the intensity of insects' feeding was determined calculating the size of eaten area of discs in each experimental combination as a difference between the surface of standard and tested disc. Later on obtained data were submitted to statistical analyses.

Results of experiments concerning food choice are shown in Table 1. As it can be seen the most intensive feeding both by larvae and beetles of Colorado potato beetle was observed in control combinations. In case of control combination with larvae among 100 discs only one stayed untouched and the mean eaten area of disc was 149.9 mm<sup>2</sup>, what makes 53.3% of initial disc surface. Beetles fed less intensive and in this case the number of untouched discs was also low (7 out of 100) and the mean eaten area of a disc amounted about 64 mm<sup>2</sup> what makes about 31%.

The situation was completely different in nochoice tests where all discs were treated with quillaja saponins extract. Insects having no choice were forced to feed on treated leaf discs. Both larvae and beetles clearly avoided feeding on such discs. Larvae left as many as 48 discs out of 100 discs untouched what makes almost half of all tested discs, and those discs on which larvae fed were damaged in low degree. The mean eaten area of one disc amounted only to about 9 mm<sup>2</sup> that makes 4.5% of initial disc area. Beetles fed on treated discs even more unwillingly: more than a half (66) of discs stayed untouched and the eaten area of disc was also small (8.5 mm<sup>2</sup>).

In choice tests the difference between control discs and discs treated with saponin extract of quillaja was evident. All control discs (50) were damaged by larvae feeding and mean eaten area of one disc amounted to about 147 mm<sup>2</sup> (about 52% of standard disc area), while among 50 treated discs 16 stayed untouched and mean eaten area was only about 14 mm<sup>2</sup> – otherwise 10 times less than in control treatment, that makes hardly 5%. In case of beetles results were similar.

Adults more willingly fed on control discs eating an average 71 mm<sup>2</sup> of disc, what makes about 25% of the area of standard disc and among 50 discs only 3 remained untouched. Instead, in combination with discs treated with saponin extract results were similar like in combination where all discs were treated (without choice). Major-

ity of discs (38 out of 50) remained untouched and mean eaten area of one disc was only 1.5% (4.1 mm<sup>2</sup>) area of standard disc.

All data obtained by measurements of discs residues in choice and nochoice tests were submitted to statistical analysis of variance using ANOVA test and U-test (Mann-Whitney) for larvae and beetles separately. Results of statistical analysis indicated in all cases statistically significant differences between tested control and treated combinations both for larvae and beetles.

Table 1. Intensity of feeding of Colorado potato beetle on control and treated potato leaf discs in choice and no choice tests

Combination	Initial number of leaf discs	Number of untouched leaf discs	Mean eaten area of one disc in mm <sup>2</sup>	Percentage of eaten area of one disc
LARVAE				
Control	100	1	149.9	53.5
No choice tests: Quillaja	100	48	9.3	4.5
Choice tests:				
Control	50	0	146.7	52.4
Quillaja	50	16	14.2	5.1
BEETLES				
Control	100	7	64.4	30.9
No choice tests: Quillaja	100	66	8.5	4.1
Choice tests:				
Control	50	3	70.9	25.3
Quillaja	50	38	4.1	1.5

Results obtained in experiments concerning the development of larvae showed great differences in insects' survival on treated and untreated leaves. Results are shown in Figure 1. In control combinations larvae fed and developed normally, but among larvae feeding on potato leaves treated with quillaja extract a very high mortality was observed. An average in 2 series of experiments, already on third day of rearing larvae on treated leaves, the number of insects was decreased almost by half and on 6th-day only single insects stayed alive in comparison to control leaves where larvae still fed and were in good condition. So, it can be stated that quillaja extract present in larvae food showed considerable toxicity in relation to young larvae of Colorado potato beetle causing inhibition of their development and their high mortality in short time.

The observations of larvae weight gain also confirmed the inhibition of the development of larvae feeding on treated leaves. Results obtained are presented in Figure 2. Average initial weight of one larva was very similar in both combinations, but as it could be seen in Figure 2 while the weight gain of larvae feeding on control leaves was steady, in case of larvae feeding on treated leaves constant decrease of their weight was observed from the beginning of the rearing.

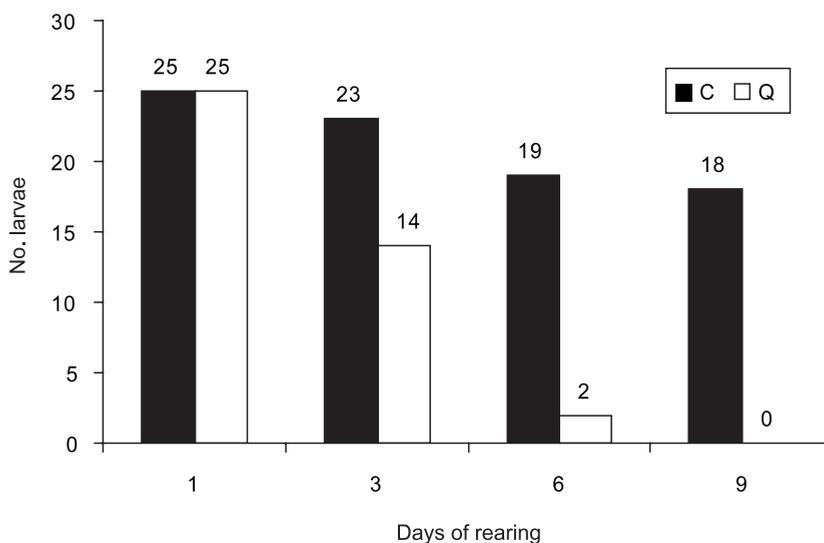


Fig. 1. Survival of Colorado potato beetle larvae on control (C) and treated with quillaja saponins extract (Q) potato leaves

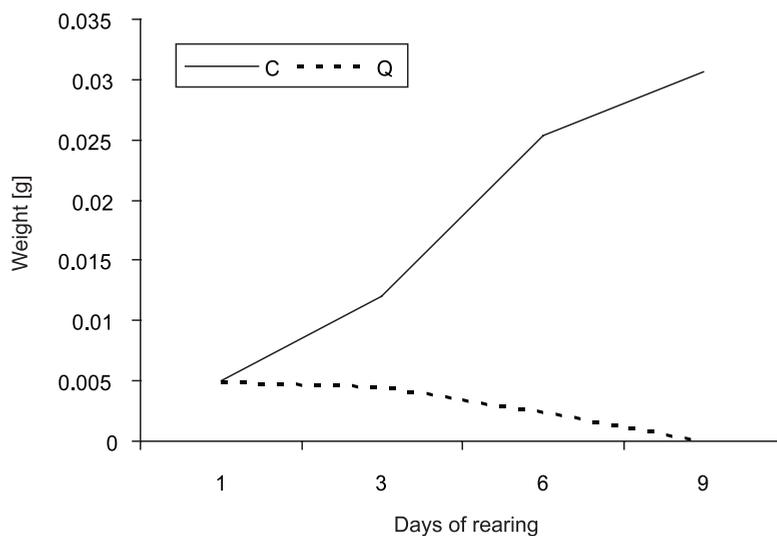


Fig. 2. Changes in weight gain of Colorado potato beetle larvae feeding on control (C) and treated with quillaja saponin extract (Q) potato leaves - mean of 2 series

In general, in all experiments with food choice the most intensive feeding both larvae and beetles of Colorado potato beetle was observed in control combinations. Insects having choice always fed more willingly on control leaves, and that difference was evident. If insects were forced to feed on leaf discs treated with quillaja saponins extract, it was observed that their feeding was significantly restricted in case of both larvae and beetles. Especially larvae avoided feeding on such discs and they left al-

most half of all treated discs untouched. It speaks for antifeedant activity of quillaja saponins and confirms results obtained earlier by different authors showing such properties of saponins originated from other plants in relation to CPB and other insects (Nozzolillo et al. 1997; Waligóra 1998; Adel et al. 2000; Szczepanik et al. 2001).

Results obtained by us in experiments concerning the development of larvae showed strong effect of quillaja saponins on feeding behavior and development of larvae of CPB. Applied extract influenced the duration of time of larvae feeding. In combinations with saponins extract feeding was restricted while in control combinations larvae fed and developed normally. Among larvae feeding on potato leaves treated with quillaja extract a very high mortality was observed in short time (6 days). Development of larvae feeding on treated leaves was evidently restrained what was expressed in their weight gain in comparison to control larvae. In our experiments the weight gain of larvae feeding on control leaves was steady, while in case of larvae feeding on treated leaves constant decrease of their weight was observed from the beginning of the rearing. In these experiments we used 2nd instar larvae and nevertheless their mortality was high. At this concentration of saponins rearing of younger larvae was impossible, because they all died within one day.

So, it can be stated that saponins from quillaja bark similar like other saponins also showed considerable toxicity in relation to larvae of Colorado potato beetle causing inhibition of their development and their high mortality in short time. As it can be seen wide range of saponins originated from different plants act in the same way in relation to insects (Birk 1969; Roof 1972; Horber 1972). However, the question of the mechanism of the saponins' effect on the insects is still without the answer.

## CONCLUSIONS

1. Tested saponins extract originated from quillaja bark had influenced Colorado potato beetle feeding similar like tested earlier saponins extracts from other plants – causing considerably reduction of food consumption of both larvae and beetles of this pest.
2. It did not reveal repellent action towards this insect but rather it acts as antifeedant.
3. Beetles of this insect seemed to be less susceptible than larvae for the presence of saponins in the food that confirmed our previous observations concerning saponins extracted from other plants.

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**POLISH SUMMARY****BADANIE AKTYWNOŚCI EKSTRAKTU SAPONINOWEGO Z KORY  
MYDŁOKI WŁAŚCIWEJ (*QUILLAJA SAPONARIA* MOLINA) W STOSUNKU  
DO STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY)**

Badano wpływ ekstraktu saponinowego z kory mydłoki właściwej (*Quillaja saponaria* Molina) na wybór pokarmu i rozwój stonki ziemniaczanej (*Leptinotarsa decemlineata* Say) prowadząc testy na wybór pokarmu z zastosowaniem krążków wyciętych z liści ziemniaka traktowanych lub nietraktowanych (kontrolnych) badanym ekstraktem. Obserwowano wpływ obecności saponin w pokarmie stonki na wybór pokarmu przez larwy i chrząszcze tego owada oraz wpływ badanego ekstraktu na rozwój larw stonki. Stwierdzono, podobnie jak w przypadku saponin pochodzących z innych roślin, że związki te powodowały znaczne ograniczenie żerowania stonki ziemniaczanej oraz zakłócenia w rozwoju larw tego szkodnika.