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Plant Physiology

Insecticidal Action of Chitin-Binding Mistletoe (*Viscum album* L.) Fruit Lectins against *Apamea sordens* Hufn. and *Pyrausta nubilalis* Hb. (Lepidoptera: Noctuidae)

Nino Keburia*, Ekaterine Khurtsidze*, Mariam Gaidamashvili*

* *I. Javakhishvili Tbilisi State University*

(Presented by Academy Member Nugzar Aleksidze)

ABSTRACT. Chitin-binding lectins from mistletoe (*Viscum album* L.) fruit were examined for insecticidal activity against *Apamea sordens* Hufn. and *Pyrausta nubilalis* Hb. (Lepidoptera, Noctuidae) larvae at different stages of development. Lectins affected larval survival and showed delay of pupation time. The results demonstrate that mistletoe chitin-binding lectins have obvious anti-nutritive effects on Lepidoptera insects. *© 2010 Bull. Georg. Natl. Acad. Sci.*

Key words: *Lepidoptera, mistletoe, chitin-binding lectin, insecticidal activity.*

Insects are in competition with human agro-industry. Every day, 1/6 of the world's agricultural production is consumed by insects, especially in those countries which are the most dependent on agriculture for their subsistence or for the revenue it generates for health and development expenses. Lepidoptera are the most diverse group of animals in the world and they are practically all herbivores. Among the polyphagous Lepidoptera insects *Apamea sordens* (Noctuidae) and *Pyrausta nubilalis* (Pyralididae) are the largest in the order and are responsible for significant post-harvest loss of agricultural production. Intensive use of agrichemicals for pest control results in increased pest resistance and subsequent growth of pesticides applied to the fields. Due to the environmental concerns of pesticide use and limited list of effective alternatives it is therefore urgent to develop novel biopesticides from plants and other natural sources that have low mammalian and environmental toxicity for crops.

The insecticidal activity of plant lectins against a large array of insect species belonging to the Coleoptera, Homoptera, Diptera and Lepidoptera order has been well documented [1-3]. Insecticidal activity was found to be associated mostly with the two main groups of plant lectins: monocot mannose-binding and chitin-binding lectin groups. *Galanthus nivalis* bulb lectin and wheat germ agglutinin are the best characterized representatives of this group which is increasingly used in the development of transgenic crops [4].

In this paper we analyze chitin-binding lectins (VAC1 and VAC2) from mistletoe (*Viscum album* L.) fruits for insecticidal activity toward *Apamea sordens* Hufn. (rustic shoulder-knot moth) and *Pyrausta nubilalis* Hb. larvae.

The fruits of European mistletoe were harvested in the mountainous region of Eastern Georgia, in winter (December-February) and stored at -15°C until use. VAC1 and VAC2 lectins were prepared as described with some modifications [5]. The ripe fruits were homogenized in medium consisting of 0.05 M Na-acetate buffer, pH 4.5, at ratio 1:3 (g/ml). The extracts were centrifuged at 5000g for 15 min; supernatant was filtered through Watman GF/c filter. The soluble protein fractions were purified by affinity chromatography on the agarose (Serva) and chitin (Sigma) sorbents, dialyzed and stored until use.

Table 1.

The effects of mistletoe lectins on the development of *Apamea sordens* larvae.

Treatment	Days to reach pupation	Rate of pupation (%)	Pupae period (days)	Pupae weight (mg)	Rate of adults emerging from pupa (%)	Insect survival (%)
VAC1	22±2	36.2%	13±2	254±50	54.3%	18.3%
VAC2	25±1	10%	0%	170±5	0%	0%
control	23±1	411.%	13±2	254±50	41.7%	17.25%

The larvae of *Pyrausta nubilalis* Hb. (e.g. *Ostrinia nubilalis* Hbn.) and *Hadena basilinea* Schiff. (F.) (*Apamea sordens* Hufn.) were obtained from Zugdidi (West Georgia) and Khashuri (East Georgia). Larval cultures were reared at 25±1°C and relative humidity of 65-75%, under a 16/8 light regime. Insect larvae were reared on a control and experimental diet with or without lectin, respectively. The lectins were incorporated into natural diet daily at a level of 5, 10 and 20 mg final doses. 10-15 larvae were used per treatment. Insect survival was estimated daily, the weights of larvae and pupae were measured and the duration of developmental stages was determined. In insect bioassays the effects of VAC1 and VAC2 lectins on the development and survival of *Apamea sordens* and *Pyrausta nubilalis* larvae were examined.

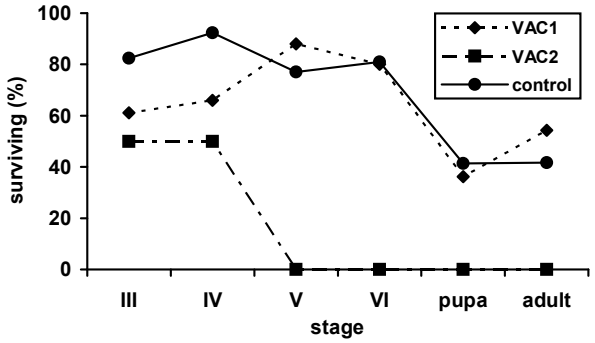

Fig. 1. Effect of VAC1, VAC2 lectins on the survival of *A. sordens* at different stages of larvae development.

The effects of VAC1 and VAC2 on the growth and mortality of *Apamea sordens* larvae is shown in Table 1. The rates of adults successfully emerging from pupae fed on VAC1 and VAC2 were 54.3% and 0%, respectively. This first rate was not lower than those of control insects (41.7%). Final instar larvae and pupae fed on VAC1 showed the same surviving rates as the control insects. In contrast, VAC2 showed zero survival of larvae. VAC2 increased the period of larva pupation (25±1 days). The rate of larvae pupation was nearly four times lower (10%) than those of the control insects.

Insecticidal effect of VAC1, VAC2 lectins on the different developmental stages of larvae is shown in Fig. 1. Generally, in all experimental groups larval mortality observed was higher than that of the control group. The mortality of third and fourth instar larvae fed on VAC1 and VAC2 was 36% and 50% respectively. The survival of fourth stage larvae fed on VAC2 dramatically decreased as compared to that of control insects (7%). At the final stages of development both lectins did not show significant influence on larval survival. The results suggest that the influence of lectins was more evident at the early stages of larval development. Apparently, this is related to the greater sensitivity of glycosylated gut structures of young insects to carbohydrate-binding plant lectins.

Short-term bioassays on *P. nubilalis* showed 67% and 50% of larval survival when fed on VAC1 and VAC2 lectins respectively. This showed a much lower rate than those of control insects (80%). Prolongation of the last instar and dalay of pupation was observed in both experimental groups.

In conclusion, obvious insecticidal activity of VAC lectins was observed on both *Apamea sordens* Hufn. and *Pyrausta nubilalis* Hb. larvae. Lectins affected the larval development as well as their survival and showed delayed pupation time for both insects. It appears that binding to insect gut structures and apparent resistance to proteolytic degradation by the insect digestive enzymes are two basic prerequisites for lectins to exert their deleterious effect on insects.

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მცენარეთა ფიზიოლოგია

ფითრის (*Viscum album* L.) ნაყოფის ქიტინ-დამაკავშირებელი ლექტინების ინსექტიციდური თვისებები

ნ. ქებურია*, ე. ხურციძე*, მ. გაიდამაშვილი*

* *ი. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი*

(წარმოდგენილია აკადემიის წევრის ნ. ალექსიძის მიერ)

ნაშრომში შესწავლილია ფითრის (*Viscum album* L.) ქიტინ-სპეციფიკური ლექტინების (VAC1, VAC2) ინსექტიციდური აქტივობა *Apamea sordens* Hufn. და *Pyrausta nubilalis* Hb. (Lepidoptera, Noctuidae) ლარვების მიმართ განვითარების ციკლის სხვადასხვა საფეხურზე და გამოვლენილია მათი ანტიკვებითი ეფექტი ლარვების ფიზიოლოგიურ განვითარებასა და სიცოცხლისუნარიანობაზე. ნაჩვენებია, რომ VAC1 და VAC2 ლექტინებით მკვებავი ლარვების გადარჩენის ხარისხი საშუალოდ 2,5-ჯერ ჩამორჩებოდა საკონტროლო მონაცემებს. ლექტინების ინსექტიციდური აქტივობა მეტად ვლინდებოდა ლარვების ფიზიოლოგიური ციკლის ადრეულ ეტაპებზე. გამოთქმულია ვარაუდი, რომ ფითრის ნაყოფის ქიტინ-დამაკავშირებელი ლექტინები ტოქსიკურ ეფექტს ახდენენ მწერების განვითარებაზე, რაც იძლევა მავნე მწერების წინააღმდეგ მათი გამოყენების შესაძლებლობას.

აღნიშნული პროექტი განხორციელდა საქართველოს ეროვნული სამეცნიერო ფონდის ფინანსური ხელშეწყობით (გრანტი № GNSF/ST08/8-519). წინამდებარე პუბლიკაციაში გამოთქმული ნებისმიერი აზრი ეკუთვნის ავტორს და შესაძლოა არ ასახავდეს საქართველოს ეროვნული სამეცნიერო ფონდის შეხედულებებს.

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