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Effect of some proteins on biology of grain aphid (*Sitobion avenae* /F./)

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Abstract: Extensive studies have been carried out to identify plant proteins with insecticidal properties towards insects. The paper describes a test for screening toxicity and growth inhibition of five commercially available proteins such as: bromelain, honey bee venom, two lectins-wheat germ agglutinin (WGA), concanavalin A (Con A) and lysozyme in artificial diets on *Sitobion avenae* /F./. Grain aphid proved to be sensitive to all tested proteins. Among the tested proteins concanavalin A and wheat germ agglutinin had the highest negative influence on feeding, reduced weight and increased mortality of tested aphids independently from the dose. It suggested that this group of the proteins have an insecticidal activity and is a good candidate for control of the insect pests.

Keywords: feeding, survival, toxicity, aphid , proteins

INTRODUCTION

Previous studies suggested that the diet of aphids contained low-protein phloem sap [1] and aphid salivary secretions contain no proteases [2]. Therefore, the studies on aphid nutrition largely ignored the protein components of the food source and focused on small molecular weight compounds such as free amino acids, sugars, minerals or vitamins. However, recent research has shown that plants can accumulate proteins up in the phloem sap to high concentration [3] and indicates the occurrence of proteases in midgut of phloem-feeding insects [4]. However, it should also be emphasized that the nutritional value of proteins

is only one of the factors affecting aphid-plant relationships. Many proteins from phloem sap are connected with their functions in wound and defence reactions of plants against insects. Moreover, it has been found that phloem is not the only tissue with which aphids interact. Proteins also occur in the plant cell walls, either naturally or as a result of aphid infestation, and many of such proteins are suspected to affect aphid feeding activities [5]. Therefore, extensive studies have been carried out to identify plant proteins with insecticidal properties towards insects. Many of them are being tested for repellent, deterrent or lethal effect against aphids. It would be useful to develop novel biotechnological strategies to enhance the resistance of crop plants against phloem-feeding insects. Allelopathic substances are common in ecosystem [6].

The aim of the present study was to examine the effects of some commercial proteins which belong to different protein classes on behaviour, growth and mortality of grain aphid (*Sitobion avenae* /F./). The following proteins were used in the test: bromelain (BRO, protease), honeybee venom (VAM, venom), wheat germ agglutinin (WGA, lectin), concanavaline A (Con A, lectin) and lysozyme (LYS, glycosidase).

MATERIAL AND METHODS

Laboratory experiments were performed at 20 ±3 °C temperature and at 65 ±5% humidity according to the procedure described by Khan and Saxena [7] for insects with stinging-suctorial mouth apparatus system.

Choice-tests were used in order to describe an influence of studied proteins and their concentrations on the behaviour of winged and wingless adults and larvae (L_2-L_3). Liquid diets contained 50 $\mu g \cdot cm^{-3}$ and 250 $\mu g \cdot cm^{-3}$ proteins solutions in 20% sucrose was tested 50 mm^3 of each of the tested individual proteins or 50 mm^3 of 20% sucrose solution (control) was introduced between two M Parafilm® membranes and situated on plexiglass rings (h = 0.3 cm, Ø = 1 cm). 30 in numbers of each morph of grain aphid were used. Dishes were placed on the edge of Petri dish, covered with filter paper dripped with redistilled water. After 1 and 48 hours of the experiment aphids were counted on the parafilm membrane containing tested concentration of proteins and on the control parafilm membrane (containing 20% sucrose solutions).

Feeding tests were performed in order to investigate the antibiotic activity of the tested concentration of proteins on *S. avenae.* Seven days old winter triticale cultivar Dagro seedlings susceptible to grain aphid were used. Seedlings without roots were put into test-tubes with 2 cm^3 of 50 $\mu g \cdot cm^{-3}$ and 250 $\mu g \cdot cm^{-3}$ proteins

solutions or in water (control). Test-tubes were placed in beakers and covered with plexiglass isolator in the shape of a cylinder (h = 20 cm, Ø = 8 cm). Seven wingless females of grain aphid, previously weighed, were put on each seedling. After 48 h of feeding aphids were weighed again. The quantity of ingested food was calculated according to Khan's and Saxena's [7]:

$$\text{Food ingested} = W_1 \times \frac{C_1 - C_2}{C_1} + W_2 - W_1$$

where:

W_1- initial aphid weight (mg) before feeding experiment,

W_2- final aphid weight (mg) after 48 hours of feeding on the seedling placed inside the test-tube with tested protein solution,

C_1 - initial aphid weight (mg) before putting it on the seedling placed in water (control),

C_2 - final aphid weight (mg) after 48 hours of feeding on the seedling placed in water.

The effect of tested proteins solutions on mortality of *S. avenae* was assessed in the survival tests. The life length (in days) of wingless females feeding on Dagro winter triticale seedlings placed for 14 days in tested proteins solutions or on the seedlings abosorbing water (control) were recorded.

All the entomological tests were done in three independent replicates. The influence of the studied proteins on behaviour, feeding and survival rate of testing grain aphid development phases were subjected to analysis of variance and significance of the differences was tested using Duncan Multiple Range Test at level $P \leq 0.05$.

RESULTS AND DISCUSSION

The number of aphids on the parafilm membrane containing tested concentration of proteins counted one hour from the onset of the test was used to indentify ,,short-term'' phagostimulating properties of proteins. On the basis of the rate of settling on diets 1 h after the deposition of larvae, winged and wingless females, none of the proteins tested exerted deterrent effect on the behaviour of *S. avenae* (Tables 1and 2). Similar results were obtained by Rahbe and Febvay [5] who showed that none of the proteins tested (twenty five proteins) had deterrent effect to pea aphid (*Acyrthosiphon pisum* H). It is consistent with the thesis formed by Yamashita *et al*., [8] that proteins are commonly devoid of phagostimulatory properties, except for some very specific compounds such as

polipeptidic sweeteners or taste modifiers. However, the results of the choice test indicated that the reaction of insects to dietary proteins changed after 48 hours of aphids feeding. All of the proteins tested exert deterrent effect on behaviour of *S. avenae* (Tables 1 and 2). Among the tested proteins, Con A and WGA had the biggest negative influence on the feeding of development phases of grain aphid. In addition, it was found that the protein concentration of 250 $\mu g \cdot cm^{-3}$ affected the aphid behaviour more than 50 $\mu g \cdot cm^{-3}$. The experiments further showed that tested proteins had the most negative effect on the larvae phases.The differences in the choice between 50 $\mu g \cdot cm^{-3}$ and 250 $\mu g \cdot cm^{-3}$ concentration of proteins found among *S. avenae* at different development phases were statistically significant. The obtained results suggested that the tested proteins did not have „short-term" phagostimulatory status but their negative effect on the behaviour of grain aphid increased when the feeding time of the grain aphid was longer.

Table 1. Number of the grain aphids found on diets contained 50 $\mu g \cdot cm^{-3}$ of proteins after 1 and 48 h of the exposure

Treatments	Morph of *S. avenae*					
	Larvae		Wingless females		Winged females	
	1 h	48 h	1 h	48 h	1 h	48 h
Bromelain (BRO)	2.00 a	1.00 b	6.00 a	2.00 b	6.33 a	2.33 b
Honey Bee Venom (VAM)	1.66 a	1.33 b	6.33 a	2.33 b	6.66 a	3.33 b
Wheat Germ Agglutinin (WGA)	1.66a	0.66 c	5.55 a	1.33 c	5.55 a	1.00 c
Concanavaline A (Con A)	2.00 a	0.33 c	5.33 a	0.66 c	5.33a	0.66 c
Lysozyme (LYS)	2.33 a	1.66 b	6.66 a	2.00 b	5.33 a	2.33 b
Control	2.00 a	5.33 a	6.00 a	4.00 a	6.00	5.33 a

Values in columns followed by various letters are significantly different at $P \leq 0.05$ (Duncan's test).

Table 2. Number of the grain aphids found on diets contained 250 $\mu g \cdot cm^{-3}$ of proteins after 1 and 48 h of the exposure

Treatments	Morph of *S. avenae*					
	Larvae		Wingless females		Winged females	
	1 h	48 h	1 h	48 h	1 h	48 h
Bromelain (BRO)	2.00 a	0.66 c	2.00 a	1.00 b	2.33 a	1.00 b
Honey Bee Venom (VAM)	2.33 a	1.00 b	2.33 a	1.33 b	2.66 a	1.33 b
Wheat Germ Agglutinin (WGA)	2.00 a	0.33 c	2.00 a	0.66 c	2.00 a	0.66 c
Concanavaline A (Con A)	2.00 a	0.33 c	2.00 a	0.33 c	2.00 a	0.33 c
Lysozyme (LYS)	2.33 a	1,00 b	2.33 a	1.66 b	2.66 a	1.33 b
Control	2.33 a	4.00 a	2.66 a	5.33 a	2.33 a	4.00 a

Values in columns followed by various letters are significantly different at $P \leq 0.05$ (Duncan's test).

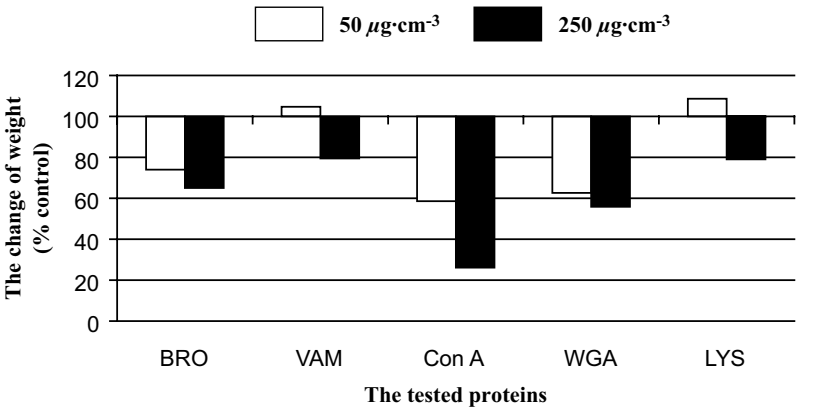

Figure 1. An influence of the tested proteins on weight of the grain aphid wingless females, after 48 h of feeding.

Moreover, the results of the entomological tests showed that almost all of the tested proteins, especially Con A and WGA had a negative influence on the quantity of ingested food (Figure 1) and reduced the weight of wingless females after 48 hours of feeding (Figure 2). Only LYS and VAM had at the lowest dose a positive influence on both of these rates. The obvious differences in toxicity between Con A and WGA can be connected with their sugar-binding specificity. Concanavaline A is glucose and mannose-binding lectin but wheat germ agglutinin has an affinity for N-acetylglucosamine. Similar results were obtained

in survival tests (Table 3). It was statistically proved that all of the tested proteins (except LYS) reduced the aphid survival rate, among which Con A and WGA were those reduced the aphid survival most strongly. These results are similar to those of Rahbe's and Febvay's [5] who showed that plant lectins particularly concanavalin A, displayed significant toxicity and growth inhibition of *A. pisum*. Lysozyme and honeybee venom, on the other hand had growth stimulatory effect at the lowest dose and a slightly impairing growth at a high dose.

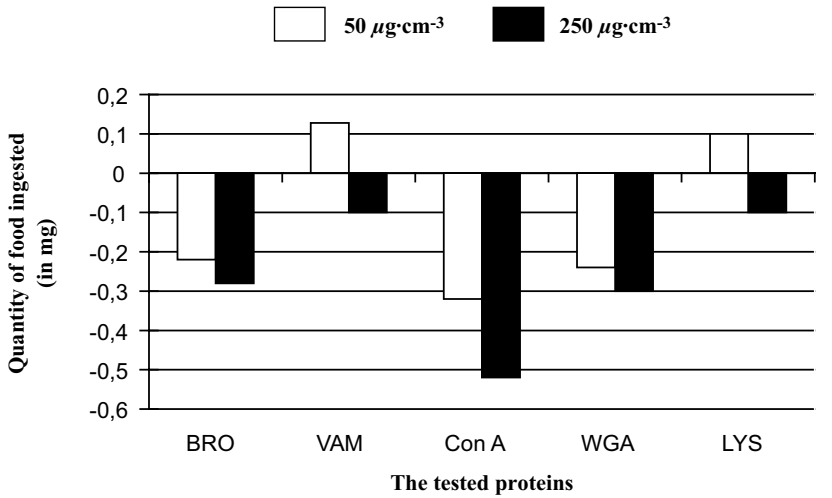

Figure 2. An influence of the tested proteins on quantity of food ingested by the grain aphid wingless females, after 48 h of feeding.

Table 3. Effect of 250 $\mu g \cdot cm^{-3}$ concentrations of the proteins on survival of the grain aphid wingless females

Treatments	Survival rate (in days)
Lysozyme (LYS)	10.0 a
Control	9.00 b
Honey Bee Venom (VAM)	8.00 c
Bromelain (BRO)	6.66 d
Wheat Germ Agglutinin (WGA)	6.00 d
Concanavaline A (Con A)	4.00 e

Values in columns followed by various letters are significantly different at $P \leq 0.05$ (Duncan's test).

The same tendency was observed in case of bromelain. This protein was toxic to both pea aphid [5] and grain aphid. One hypothesis of this toxicity implies the

degradation of luminal protein structures (e.g. intestinal membrane glycoproteins) by ingested proteases. In conclusion, the data presented here suggest that plant proteins can be toxic to grain aphid but it depends on their concentration. Moreover, it should be added that plant lectins have insecticidal activity and are a good candidate for control of the insect pests. Recently numerous reports have been published on the insecticidal activity of plant lectins against many insect pests which belong to the orders of *Lepidoptera*, *Coleoptera, Diptera* and *Homoptera* [9-11]. However, studies indicated that the observed entomotoxic or insecticidal activity are specific and related to the fact that plant lectins are a complex composite of multiple families of evolutionary related proteins with markedly different biochemical and physicochemical properties, carbohydrate-binding specificity and biological activities [12]. Studies on chemistry and mechanisms of allelopathic interactions are needed.

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