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ORIGINAL PAPER

The effects of plant extracts on apple aphid (*Homoptera: Aphis pomi* De Geer) under laboratory conditions

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Abstract

The effects of different plant extracts on apple aphid (Aphis pomi) mortality were compared under laboratory conditions. The apple aphid is one of the most resistant pests against insecticides throughout the Central Europe. The selection of insecticide-resistance pest biotypes can be delayed by decreasing the frequency and intensity of genetic selection. This might be accomplished by applying insecticides only when and where absolutely necessary and in minimum acceptable amount or to involve methods and use different plant extracts as biopesticides.

In our experiment extracts from the following plants were used: Azaron 3% and 6% (Acorus calamus), Arnicin 3% and 6% (Arnica montana), Allicin 3% and 6% (Allium sativum), Hellebrin 3% and 6% (Helleborus purpurascens), Populin 3% and 6% (Populus nigra) and distillate water as control.

All extracts with 6% concentrations has significant effects and the mortality was high compared with control. These suggest that the chemical components of these extracts may be used as biopesticides in integrated and biological pest management control.

Keywords biopesticides, crop disaster, insecticide resistance, morality, pest management

Introduction

Since 1914, when resistance to lime sulphur spray in the San Jose scale was firs reported, over 400 cases of insecticide resistance have been documented, a number of these resulting in crop disaster [1]. The selection of insecticide-resistance pest biotypes can be delayed by decreasing the frequency and intensity of genetic selection. This might be accomplished by applying insecticides only when and where absolutely necessary and in minimum acceptable amount. Rate of change to pesticide-resistance genotypes may be reduced where cultural and biological tactics are includes in management systems to reduce the frequency of damaging infestations and the number of pesticidal applications [2, 3]. Deciduous tree fruits, especially apples have been fruitful systems from many countries throughout Europe. Apples are perennials, having ecological diversity and habitat continuity, including microhabitat complexity and food features for variety of arthropods. Fortuitously, development of insecticide resistance among the major insect pest of apples (codling moth, *Cydia pomonella*, apple maggot *Rhagoletis pomonella*, plum curculio, *Conotrachelus nenuphar*, apple aphid, *Aphis pomi*) has not been studied during the past 20 year, even under intensive applications of organophosphate-based insecticides. Resistance allows some species to commonly persist and exert continuing controlling actions in commercial orchards where a variety of pesticides are widely used. It has raised questions regarding to introduction of new pesticides into apple orchards, because of its possible insecticide adapted one that is relatively stable with regard to natural control [4, 5]. There were no references about the uses of the plant extract used by us in aphid pest control.

Aphis pomi

The adult is 1.5-2.0 mm long; body oval and relatively rounded; green with black siphunculi and cauda; legs and antennae paler and tipped with brown. Winged adult: thorax black; abdomen green with 3 pairs of black lateral circular spots on the anterior abdominal segments and a semicircular spot in front and behind each siphunculus. Egg: greenish-yellow to green, rapidly turning to shiny black. Winged oviparous females and apterous males appear in October and November. After mating, each female lays eggs on the twigs, preferably at the top of current year's growths. These eggs are sometimes grouped in large numbers, unlike those of other aphid pests [6].
Host plants: apple, more rarely pear, hawthorn (*Crataegus oxyacantha*), medlar (*Mespilus*), quince, rowan or mountain ash (*Sorbus*), rose (*Rosa*) and spiraea (*Spiraea*). Unlike most harmful aphids, *A.pomi* is an autoecious species.
Damage: Feeding punctures of fundatrices and their offspring hinder growth of the young twigs of host trees and sometimes distort them. Damage is more severe on nursery stock and seedlings. In summer, sooty moulds develop on the honeydew sometimes which is produced in large quantities [7].

Material and Methods

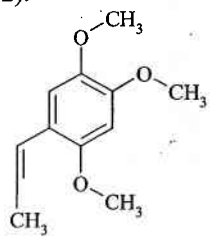
The collected plant material (stems, leaves, flowers) is dried in a ventilated oven at 45 °C for 24 H. An amount of 20.0 g of the dried plant powder is weighed in an Erlenmeyer of 100 ml to which 70 ml of hexane (purity grade 99 %) is added (the plant sample has to be submerged with solvent) for pre-extraction. The Erlenmeyer is placed in a sonicator-bath (Branson 8210 or some other type) and sonicated at a temperature 40 °C during 30 minutes. The mixture is filtered using paper filter, followed by washing the Erlenmeyer with 20 ml of hexane and then with 50 ml of hexane. The filtrate is poured in a round-bottomed flask and the solvent is concentrated (at about 11 mm Hg) up to 5-10 ml by means of rotavapor, utilizing a water bath at 40 °C. This residue is brought in a 30-ml vessel to let the solvent evaporate. The open vessel is left overnight in a well-ventilated hood in order to evaporate the last traces of the solvent in the hexane pre-extract.
Extracts from the following plants were used to study the mortality of the Aphis pomi:
Acorus calamus 3% and 6%, *Arnica montana* 3% and 6%, *Allium sativum* 3% and 6%*Helleborus purpurascens* 3% and 6% and *Populus nigra* 3% and 6%,. All extracts were compared with distillate water, used as control.
100 individual of aphides were places in four repetitions in 90 mm diameter Petri dishes. 2 ml plant extracts were introduced and the numbers of death individuals were counted in every 0, 5, 15, 30 and 60 minutes. The experiment was repeated four times for all plant extract and concentration.

Data analyses

We carried out analyses of variance (ANOVA) to determine whether there were any differences between the mortality of the *Aphis pomi* caused by different plant extract and control solution. ANOVA is a general technique, which is used to test the hypothesis that the means among two or more groups are equal, under the assumption that the sampled populations are normally distributed. If the null hypothesis (no difference among interactions) is accepted, there is an implication that no relation exists between the factor levels and the response. If a significant F-value is found for one independent variable, then this is referred to as a significant main effect. However, when two or more independent variables are considered simultaneously, there is also an interaction between the independent variables - which may or may not be significant [8]. Back-transformed means and 99% confidence limits are considered as statistically significant differences.

Results

The number of individuals killed by Azaron (1,2,4-trimethoxy-5-propenylbenzene) **-1**.-, *(Acorus calamus)* 3% compared with control was significant only after 60 seconds ($P<0.01$), while for other times these were the same with the control (Fig. 1). Analyzing the number of individuals killed by *Acorus* 6% compared with control significant differences were observed for all counting times (P<0.01) (Fig. 2).



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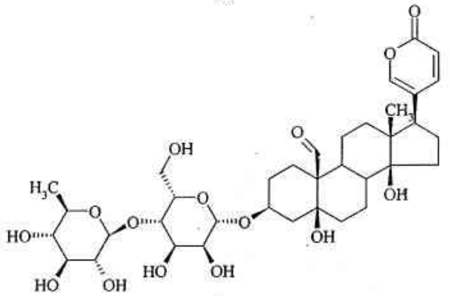

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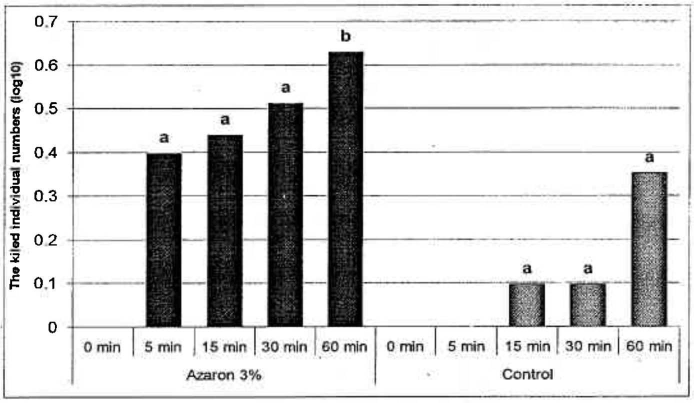


Figure 1. The number of individuals killed by Azaron (1,2,4-trimethoxy-5-propenylbenzene) 3% compared with control. Different letters = P<0.01

The plant extract Hellebrin (5-beta-Bufa-20,22-dienolide, 3-beta-((6-deoxy-4-O-beta-D-glucopyranosyl-alpha-L-mannopyranosyl)oxy)-5,14-dihydroxy-19-oxo-) **-2.-** 3% collected from *Helleborus purpurascens* has a significant effect only after 5, 15, 30 and 60 seconds compared with control (P<0.01). In the same time the mortality of the aphides was also high in control after 60 seconds (Fig. 3). The Hellebrin 6% has in the same time a significant mortality effects on aphides for all counting period (P<0.01) (Fig. 4).

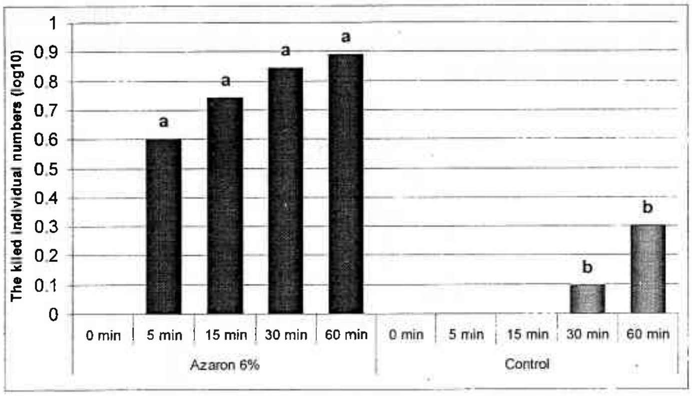


Figure 2. The number of individuals killed by Azaron (1,2,4-trimethoxy-5-propenylbenzene) 6% compared with control. Different letters = P<0.01

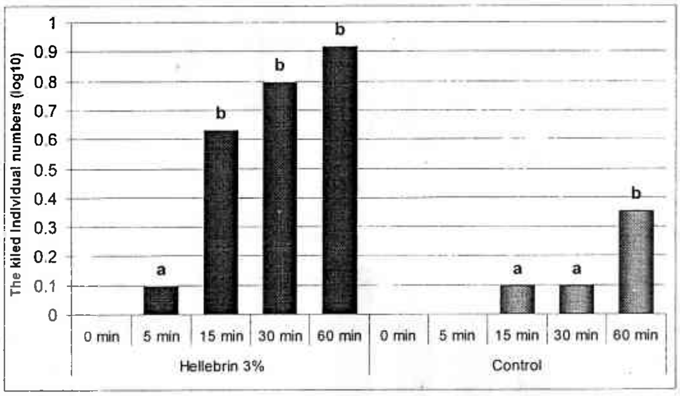


Figure 3. The number of individuals killed by Hellebrin (5-beta-Bufa-20, 22-dienolide, 3-beta-((6-deoxy-4-O-beta-D-glucopyranosyl-alpha-L-mannopyranosyl) oxy-5, 14-dihydroxy-19-oxo-) 3% compared with control. Different letters = P<0.01

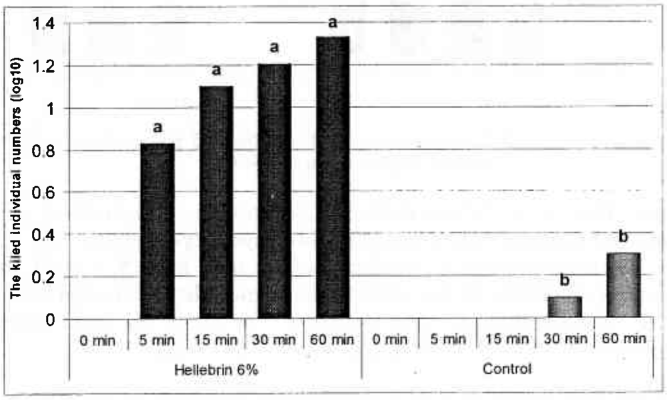
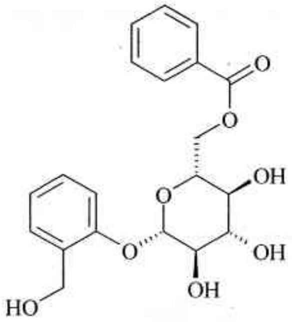


Figure 4. The number of individuals killed by Hellebrin (5-beta-Bufa-20, 22-dienolide, 3-beta-((6-deoxy-4-O-beta-D-glucopyranosyl-alpha-L-mannopyranosyl) oxy-5, 14-dihydroxy-19-oxo-) 6% compared with control. Different letters = P<0.01

The numbers of individuals killed by Populin (2-(Hydroxymethyl)phenyl-beta-D-glucopyranoside 6-benzoate) -**3**.-, (*Populus nigra*) 3% and 6% presented the similar value and were significant only after 5 minutes compared with control (P<0.01) (Fig. 5, 6).



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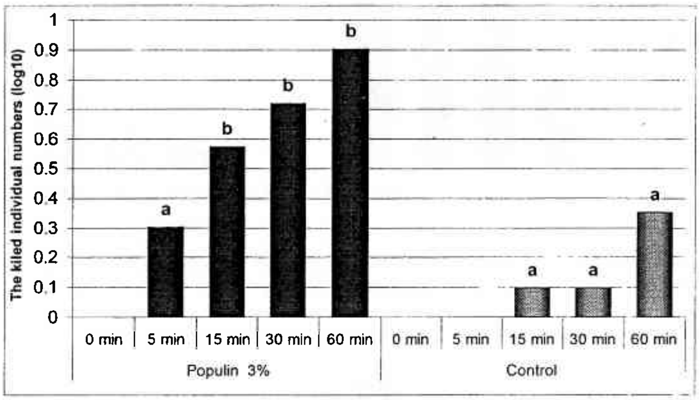


Figure 5. The number of individuals killed by Populin (2-(Hydroxymethyl) phenyl-beta-D-glucopyranoside 6-benzoate) 3% compared with control. Different letters = P<0.01

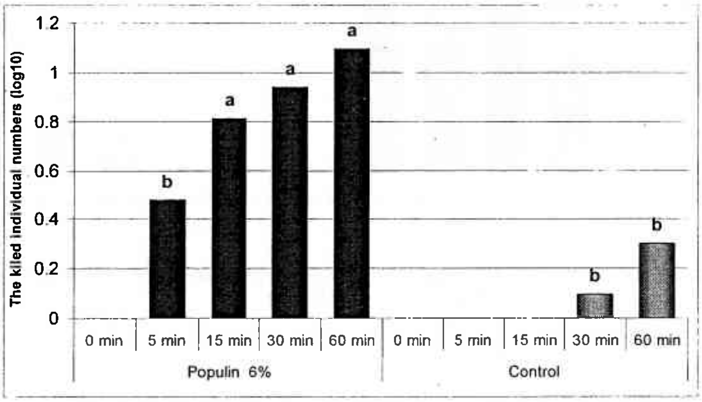
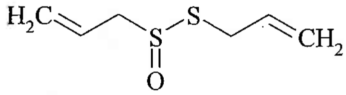


Figure 6. The number of individuals killed by Populin (2-(Hydroxymethyl) phenyl-beta-D-glucopyranoside 6-benzoate) 6% compared with control. Different letters = P<0.01

The same results were observed for Arnicin (*Arnica montana*) 3% and 6%, and Allicin (2-Propene-1-sulfinic acid, thio-, S-allyl ester) -**4.**-, (*Allium sativum*) 3% and 6 %. (The Arnica montana's essential oils contains an imperfectly known alkaloid called Arnicin). In all cases the numbers of aphid individuals were killed in a significant number only after 5 minutes (P<0.01). For the control solution we did not observed any individual killed in the firs 15 minutes (Fig. 7, 8, 9, 10).



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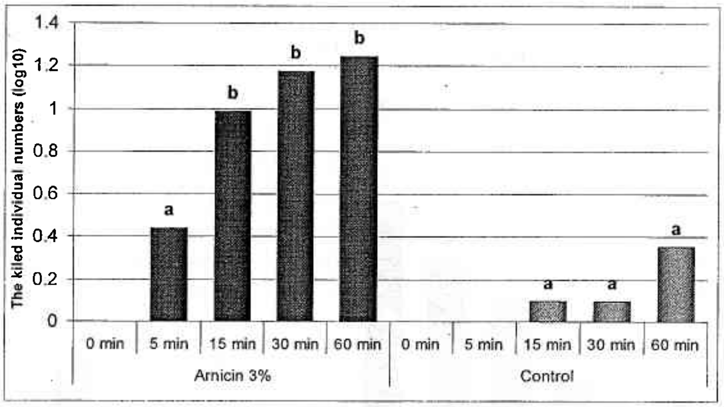

Figure 7. The number of individuals killed by Arnicin 3% compared with control. Different letters = P<0.01

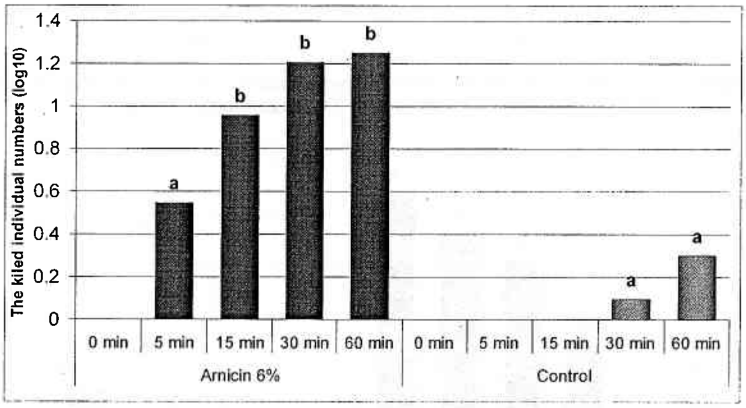

Figure 8. The number of individuals killed by Arnicin 6% compared with control. Different letters = P<0.01

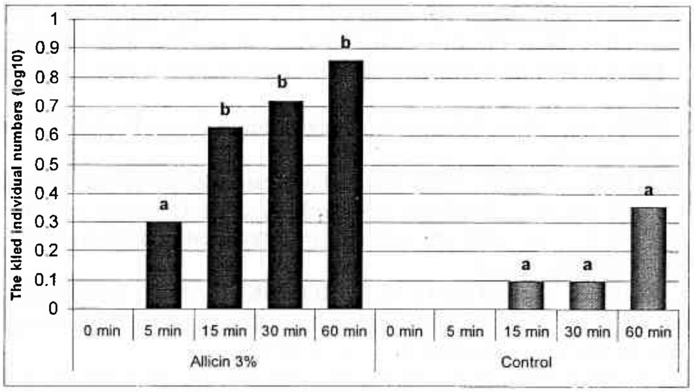

Figure 9. The number of individuals killed by Allicin (2-Propene-1-sulfinic acid, thio-, S-allyl ester) 3% compared with control. Different letters = P<0.01

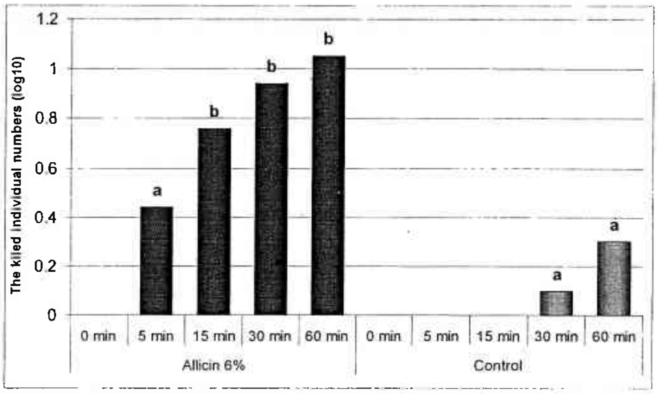

Figure 10. The number of individuals killed by Allicin (2-Propene-1-sulfinic acid, thio-, S-allyl ester) 6% compared with control. Different letters = P<0.01

Conclusions

All plant extracts used in our experiments has different mortality effects on apple aphides. We can conclude that all extracts with 6% concentrations within laboratory conditions has significant effects compared with the control. All the extracts used under laboratory conditions were tested in field experiments in vegetable crops. The results were similar, and the mortality of the apple aphid was as high as in laboratory experiment. These results suggest that the chemical component of the extract has high insecticide effects, and they could be used as biopesticides in integrated and biological pest management control. Further researches are needed to identify the theoretical and practical background of use these extracts in plant protection.

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