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## Efficacy of three natural substances against apple aphid (*Aphis pomi* De Geer, Aphididae, Homoptera) under laboratory conditions

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### ABSTRACT

In 2008 the efficacy of three environmentally friendly substances against apple aphid (*Aphis pomi*) was tested. The selected substances were cinnamic acid and two plant extracts, namely glicolic extract of comfrey (*Symphytum officinale*) and fluid extract of marigold (*Calendula officinalis*). All of the substances were tested at 0.5, 1 and 5 % concentrations. The individuals of apple aphids were collected in the organic orchard of the Biotechnical Faculty in Ljubljana; 10 aphids were then transferred to an apple tree leaf, which was previously sprinkled with water, treated with selected substance and put in a Petri dish. The efficacy was assessed at 15, 20 and 25 °C with relative humidity being 75 %. The mean corrected mortality rate was determined on the first, second and third day after treatment. In general all of the tested substances showed aphicidal properties, and the highest mean corrected mortality rates were determined on the third day at 25 °C. Only marigold extract exceeded a 50 % aphicidal efficacy at 15 °C and at 5 % concentration of the suspension used, while other treatments showed aphicidal efficacy between 4 ± 4 % (cinnamic acid, 15 °C, 0.5 %) and 39 % ± 12 % (cinnamic acid, 25 °C, 1 %). In general, the best mean efficacy (mean corrected mortality rate (15 % ± 2) % of the substances tested was found at highest concentration.

**Key words:** aphicidal efficacy, *Aphis pomi*, *Calendula officinalis*, cinnamic acid, comfrey extract, marigold extract, *Symphytum officinale*

### IZVLEČEK

#### UČINKOVITOST TREH NARAVNIH SNOVI PRI ZATIRANJU ZELENE JABLANOVE UŠI (*APHIS POMI* DE GEER, APHIDIDAE, HOMOPTERA) V LABORATORIJSKIH RAZMERAH

V letu 2008 smo preučevali delovanje treh okoljsko sprejemljivih snovi za zatiranje zelene jablanove uši (*Aphis pomi*). Izbrane snovi so bile cimetna kislina, glikolni izvleček navadnega gabeza (*Symphytum officinale*) in tekoči izvleček vrtnega ognjiča (*Calendula officinalis*). Snovi smo preizkušali pri 0,5 %, 1 % in 5 % koncentraciji. Odrasle osebkke zelene jablanove uši smo nabrali v ekološkem sadovnjaku Biotehniške fakultete v Ljubljani. Po 10 uši smo prenesli na liste jablan, ki so bili predhodno poškopljani z vodo, jih tretirali z izbrano snovjo in jih položili v petrijevke. Učinkovitost smo ugotavljali pri treh temperaturah (15, 20 in 25 °C) in relativni zračni vlagi 75 %. Smrtnost osebkov smo ugotavljali prvi, drugi in tretji dan po aplikaciji snovi. Vse snovi so pokazale aficidno delovanje, največje vrednosti povprečne korigirane smrtnosti smo določili tretji dan poskusa pri 25 °C. Le pri 5 % koncentraciji izvlečka vrtnega ognjiča smo pri 15 °C presegli 50 % stopnjo smrtnosti preučevanih žuželk, medtem ko je bila ta vrednost pri ostalih obravnavanih od 4 ± 4 % (cimetna kislina, 15 °C, 5 %) in 39 ± 12 % (cimetna kislina, 25 °C, 1 %). V poskusu je bila učinkovitost delovanja preučevanih substanc najboljša pri najvišji koncentraciji (5 %) – povprečna korigirana smrtnost (15 ± 2) %.

**Ključne besede:** *Aphis pomi*, *Calendula officinalis*, cimetna kislina, ekstrakt navadnega gabeza, ekstrakt vrtnega ognjiča, *Symphytum officinale*, učinkovitost proti ušem, zelena jablanova uš

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## 1 INTRODUCTION

Apple aphid (*Aphis pomi* De Geer) is one of the most important pests from the Aphididae family, in Slovenia (Vrabl, 1999) as well as in other European countries (Yvon *et al.*, 2000). Aphids are especially harmful on apple trees, but – being oliphagous – they can occur also on pear, quince, medlar and on some other plants. Because they suck plant juices the shoots grow weaker, they fade and the leaves do not develop. It is especially harmful in nurseries and young orchards (Haley and Hogue, 1990; Woolhouse and Harmsen, 1991; Kaakeh *et al.*, 1993). The secretion of honeydew leads to development of sooty moulds, which causes a less efficient photosynthesis and finally results in a lower marketing value (Kaakeh *et al.*, 1993).

Plant insecticides are insecticides of plant origin. They are considered natural insecticides, which originate from microorganisms (the so called microbial insecticides, produced on the basis of bacteria and viruses), plant substances and oils. Synthetic insecticides which came into use after the second world war caused the plant insecticides to become obsolete, but these latter are becoming popular again because of the problems connected with pollution, especially with the residues of phytopharmaceuticals in agricultural products and the resistance of the pests to the chemicals used. Only few plant species have so far been tested for

a potential aphicidal efficacy (Rajapakse and Van Emdem, 1997; Jaastad *et al.*, 2009).

The substances of plant origin, known to be active against pests on cultivated and wild-growing plants, are alcaloides, non-protein aminoacids, steroids, phenolic compounds, flavonoides, glycozides, glucosinolates, tannins, terpenoids and are usually secondary plant metabolites (Singh, 2010). Most plant species which are used in phytomedicine contain ingredients which inhibit the development of insects, hinder their feeding (antifeedants) or act as repellents and confusants. The substances in question act mostly as preventive agents (Rajapakse and Van Emdem, 1997; Jaastad *et al.*, 2009).

The aim of this study was to investigate the potential aphicidal efficacy of cinnamic acid, glycolic extract of comfrey and fluid extract of marigold under laboratory conditions. These substances have not been tested as potentially useful in the apple aphid control. In the case of favorable results these findings could be included in optimization of the strategy for controlling apple aphid in sustainable and ecologically friendly production of apples.

## 2 MATERIALS AND METHODS

### 2.1 Laboratory bioassay

The trial was carried out in 2008 in the Laboratory for Entomology on the Chair of Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management (Department of Agronomy, Biotechnical Faculty of the University in Ljubljana). The growing chambers (type: RK-900 CH, Kambič Laboratory equipment, Semič, Slovenia) were used to study the efficacy of three environmentally friendly substances to control apple aphid (*Aphis pomi*). The chosen substances, namely two plant extracts, i. e. glycolic extract of comfrey (*Symphytum officinale* L.) (manufacturer: Lex, Koper, Slovenia) and fluid extract of marigold (*Calendula officinalis* L.) (manufacturer: Lex, Koper, Slovenia) as well as cinnamic acid (manufacturer: Fluka, Steinheim, Germany) were tested in three concentrations (0.5, 1, and 5 %).

The specimens of apple aphids were collected in the organic orchard on the experimental field of the Biotechnical Faculty in Ljubljana (46°04'N, 14°31'E,

299 m a.s.l.). Samples were then prepared by transferring 10 aphids to each leaf (from the same location) using a brush. The leaves were previously rinsed (sprinkled) with water and dried. Specimens so prepared were sprayed by a suspension of the chosen substance and placed into a plastic Petri dish (7.0 cm in diameter) on a moist filter paper. Each Petri dish contained one leaf, they were covered by a net. The Petri dishes used were primarily intended for the studies on nontarget effect of entomopathogenic nematodes on the predators of aphids (Rojht *et al.*, 2009). Each treatment was carried out in five replications (substance tested x concentration of the suspension), the same was true also for the control treatment, where the aphids on the leaf were sprayed only by the tap water. If needed the filter paper was additionally moistured after the application. The mortality of the specimens was determined on first, second and third day after the application (the dead insects were counted).

### 2.2 Statistical analysis

A multifactor analysis of variance (ANOVA) was carried out to show the differences in mortality rates (%) of *Aphis pomi* individuals subjected to three tested substances at three different doses and at three different temperatures. Before analysis, each variable was tested for homogeneity of variance. Treatment mortalities were corrected for mortality in the untreated control treatment, using Abbott's formula (Abbott, 1925), the

arcsine square root being transformed before analysis. Duncan's multiple range test ( $p \leq 0.05$ ) was used to separate differences between treatments (Hoshmand, 2006). All statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Maryland, USA). The data are presented as untransformed means (Trdan *et al.*, 2006).

### 3 RESULTS

#### 3.1 General ANOVA analyses including all treatments

Data on analysis of the pooled results are presented in Table 1.

**Table 1:** ANOVA results for corrected mortality of adults of the apple aphid.

Source	F	Adults df	P
DAT	24.57	2	<0.0001*
suspension concentration	4.99	2	0.0073
treatment	2.31	2	0.1006
temperature	3.70	2	0.0257
replication	2.95	4	0.0205
DAT × suspension concentration	0.91	4	0.4559
DAT × treatment	0.80	4	0.5263
DAT × temperature	3.70	4	0.0058
suspension concentration × treatment	1.74	4	0.1411
suspension concentration × temperature	1.59	4	0.1766
treatment × temperature	3.51	4	0.0080
DAT × suspension concentration × treatment	0.20	8	0.9914
DAT × suspension concentration × temperature	0.52	8	0.8425
DAT × treatment × temperature	0.65	8	0.7396
suspension concentration × treatment × temperature	6.05	8	<0.0001*
DAT × suspension concentration × treatment × temperature	0.86	16	0.6217

Source of variation significant at  $\alpha=0.05$

There were no statistically significant differences in the efficacy among various potentially aphicidic substances used, while the average values for mean corrected mortality being  $11 \pm 1$ ,  $11 \pm 2$  and  $14 \pm 2$  %, respectively. But we confirmed statistically significant differences between different temperatures. The mean corrected mortality rate for the substances included in this study was the highest at 25 °C: ( $15 \pm 2$  %); but no difference was observed between 15 and 20 °C ( $10 \pm 1$  % for both temperatures). Statistically significant differences were confirmed also between concentrations of the suspensions tested. Aphid mortality was the highest ( $15 \pm 2$  %) for the highest concentration tested (5 %), less effective were substances at two lower concentrations ( $9 \pm 1$  % for 0.5 % and  $12 \pm 1$  % for 1 %, respectively). Statistically significant differences between days after treatment (DAT) were also established. The highest

aphid mortality ( $19 \pm 2$  %) was stated for 3 DAT, while 1 and 2 DAT gave statistically significantly lower mortality rates of *Aphis pomi* individuals ( $7 \pm 2$  % and  $10 \pm 2$  %, respectively).

#### 3.2 Individual analyses on different treatments

At 15 °C, 3 DAT and the highest concentration of the suspension applied, treatment with comfey extract resulted in the highest mortality rate of the apple aphids ( $51 \pm 5$  %). At 1 % concentration there were no statistically significant differences among different treatments. At the lowest concentration, treatment with comfey extract showed the lowest efficacy ( $5 \pm 4$  %) against the apple aphid (Table 2).

At 20 °C, 3 DAT and at the lowest concentration of suspension, the highest mortality rate was noted for cinnamic acid ( $25 \pm 11$  %). Treatment with comfey

extract showed the highest level of aphid mortality at 1 % concentration (32 % ± 14 %). At the highest concentration in the experiment cinnamic acid and with comfrey extract were the most effective (14 % ± 5 % and 14 % ± 3 %, respectively) (Table 2).

At 25 °C and 3 DAT there were no significant differences among different treatments at the same concentration of suspension. Comfrey extract and marigold extract gave no statistically significantly different results in the mortality rate of apple aphids at

all three suspension concentration tested and the values ranged from 18 % ± 9 % (for comfrey extract, 0.5 % suspension concentration) to 33 % ± 12 % (for comfrey extract, 5 % suspension concentration) and from 22 % ± 9 % (marigold extract, 5 % suspension concentration) to 28 % ± 11 % (marigold extract, 0.5 % suspension concentration). The highest mortality rate for the cinnamic acid treatment was obtained at both higher concentrations of suspension (39 % ± 12 % and 35 % ± 19 %, respectively) (Table 2).

**Table 2:** Mean corrected mortality (± SE) of *Aphis pomi* treated with three different concentrations of potentially aphidic substances at three different temperatures (15, 20 and 25 °C) at 3 DAT. Data are corrected for control mortality. Capital letters indicate statistically significant differences for different concentrations. Lower case letters indicate statistically significant differences for different concentrations.

tempe- rature (°C)	treatment	corrected mortality (± SE) (% ± %)		
		0.5	1	5
15	cinnamic acid	11.35 ± 8.73 Aab	6.49 ± 6.49 Aa	3.78 ± 3.78 Aa
	comfrey extr.	4.87 ± 3.67 Aa	17.84 ± 6.71 Ba	20.54 ± 8.49 Bb
	marigold extr.	19.46 ± 9.02 Ab	6.49 ± 6.49 Aa	51.35 ± 5.41 Bc
20	cinnamic acid	24.71 ± 11.11 Bb	4.70 ± 2.88 Aa	13.51 ± 5.00 Bab
	comfrey extr.	2.35 ± 2.35 Aa	7.65 ± 5.23 ABa	13.64 ± 3.42 Bb
	marigold extr.	5.29 ± 5.29 Aa	32.35 ± 14.41 Bb	7.13 ± 2.51 Aa
25	cinnamic acid	16.67 ± 4.39 Aa	38.89 ± 12.10 Ba	35.00 ± 19.43 ABa
	comfrey extr.	18.33 ± 8.68 Aa	18.33 ± 11.53 Aa	33.33 ± 11.95 Aa
	marigold extr.	27.79 ± 11.11 Aa	27.78 ± 10.20 Aa	22.22 ± 9.42 Aa

#### 4 DISCUSSION

The results of our study confirmed the assumption that the substances tested (cinnamic acid, glycolic extract of comfrey and fluid extract of marigold) possess a certain aphidic activity, but their efficacy is much less than that of the synthetic insecticides usually used to control apple aphid (Tuca *et al.*, 2009). The mean corrected mortality reached 51 % only in the case of marigold extract (a 5 % suspension at 15 °C). In a similar study (Balog *et al.*, 2007) on apple aphids 6 % suspensions of the following plant extracts were used: sweet flag (*Acorus calamus* L.), arnica (*Arnica* sp), garlic (*Allium sativum* L.), hellebore (*Helleborus* spp.) and black poplar (*Populus nigra* L.). They all showed an insecticidal efficacy, extract of hellebore being the most effective (a 100 % mortality). Other investigations proved that some mixtures containing cinnamic acid exhibit insecticidal as well as limacidal efficacy (Airey *et al.*, 1989; Watkins *et al.*, 1996). To our best knowledge no such work has been done using comfrey extracts or marigold extracts.

The efficacy of all the substances tested in our study was the highest at 25 °C, supposedly because the aphids are more active at higher temperatures (Russell and Moran, 2006) and this makes them consume more active substance compared to lower temperatures when the feeding is less intense. The same is true for the mortality being the highest on the third day after treatment at the highest suspension concentration, namely the input was the highest (Balog *et al.*, 2007; Tuca *et al.*, 2009).

The mortality of the apple aphids after application of these substances was generally quite moderate, which could be expected since the substances in question are ecologically friendly and more acceptable (Rajapakse and Van Emdem, 1997). The study showed that these substances have a potential to control the apple aphid, but their application should be optimized before their

use could be implemented in the sustainable and ecologically friendly apple production.

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