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Effect of phenolics on the pea aphid, *Acyrthosiphon pisum* (Harris) population on *Pisum sativum* L. (Fabaceae)

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Abstract: Extensive studies have been carried out to identify plant phenolics with insecticidal properties towards insects. The subject of the study were comparison of control and infested by *Acyrthosiphon pisum* (Harris) vegetative parts of pea plants. In the pea plants six flavonol aglycones were identified: quercetin, kaempferol+RCO^-, kaempferol, tricin, apigenin+RCO^-, and apigenin. In infested plants relatively high concentration of total phenols, *o*-dihydroxyphenols and total flavonoids in comparison with control were observed. It suggests that phenolics have negative effect on insects and they are good for control of the insect pests.

Keywords: Homoptera, Aphididae, Fabaceae, *Pisum sativum*, secondary metabolites

INTRODUCTION

Bioactivity of plants depends on the presence of various chemical compounds in their tissues which would inhibit insect feeding [1, 2]. Secondary plant metabolites and their degradation products are important in all agroecosystems. The following groups of compounds, among others, are known to produce toxic effect: terpenoids and steroids, phenols, coumarins, flavonoids, tannins, alkaloids, and cyanogenic glycosides [3]. Phenolic compounds have been intensively

studied with regard to their toxicity [4-6]. They play prominent roles in plant-herbivore and plant-pathogen interactions [7].

Flavonoids are naturally occurring substances in plants [8]. In legumes flavonoids play an important role in resistance. These compounds function as preformed or inducible anti-insecticidal compounds [9], so they may serve as natural pesticides. Many of them are being tested for repellent or deterrent effect against insects [10-12]. Flavonoids can modulate the feeding behaviour of insects [13, 14, Goławska – unpublished], which are ones of the most dangerous plants pests. They are controlled mostly with insecticides, but they can show a negative effect on the environment and cause resistance in insects to some chemicals [15]. For that reason it is important to find methods of inhibiting the abundance of these pests.

It would be useful to develop novel biotechnological strategies to enhance the resistance of crop plants to phloem-feeding insects. Very little is known about toxic compounds in peas. However, little information is available on the identification of active substances in pea plants. Therefore, extensive studies have been carried out to identify plant secondary compounds with insecticidal properties towards insects.

The present working hypothesis assumes that secondary metabolites contained in pea plants show activity towards insects and that they can be used in the form of natural extracts to inhibit the harmfulness of herbivorous insects. The aim of the present research was to evaluate the concentration of phenolics from pea plants, define the effect of these compounds on development of pea aphid, *Acyrthosiphon pisum* (Harris).

MATERIALS AND METHODS

Plants

Pea (*Pisum sativum* L.) (Fabaceae) var. Tulipan cultivar was used in the experiments. Seed samples were germinated in a climate chamber, which was kept at 21 ±1 °C, L16:D8 photoperiod, and 70% r.h. The plants were grown in 7 × 7 × 9 cm plastic pots in standarised soil mixture, one plant per pot. The plants were watered regularly. No extra fertilizer was added. Aerial parts of the pea plants, which were in the vegetative stage (10 days) were harvested, freeze-dried, ground, and kept in a desiccator in darkness until analyses.

Aphids

The pea aphids used in the experiments came from a stock culture kept at the University of Podlasie in Siedlce, Poland. The aphids were reared on pea seedlings [*P. sativum* L. var. Tulipan] in an environmental chamber (21 ±1 °C, L16:D8 photoperiod, and 70% r.h.).

Phenolic compounds

The content of total free phenols, soluble in ethanol was determined after Singh et al. [16] and *o*-dihydroxyphenols concentration in the EtOH extracts was measured using Arnov's method after Leszczyński [17]. The chemical analyses were repeated three times.

Ultra-performance liquid chromatography of flavonoids

Extracts were analysed by the ultra-performance liquid chromatography (UPLC) method according to Kowalska et al. [18]. Separation of the flavonoids was done using a Waters UPLC system, consisting of a Waters Acquity Ultra Performance LC Systems with Acquity UPLC BEH and Shield RP 18. The Binary Solvent Manager was used to monitor chromatographic parameters and to process the data. The pea samples were applied to an BEH C_{18} 1.7 μm 2.1 x 50 mm column and eluted at 0.35 ml min^{-1} with a linear gradient of 0.1% acetic acid in water: 40% acetonitrile in 0.1% CH_3COOH increasing to 100:0% over 8 min. Chromatograms were registered and integrated at 350 nm. The flavonoid concentrations were calculated from the total integration area, which was 350 nm. The calibration curve prepared for kemferol glycoside was used. By the ultra-performance liquid chromatography (UPLC) extracts were analysed once.

RESULTS AND DISCUSSION

On the basis of chemical analysis it was found that infested pea plants had higher concentration of total phenolics soluble in ethanol, *o*-dihydroxyphenols and total flavonoids (Figure 1). According to literature data phenolic compounds are important factors of plant resistance to aphids [19]. They may exert a synergistic effect on aphids' behaviour, physiology and metabolism and as a result reduce the aphid population on the resistant plants [20-23].

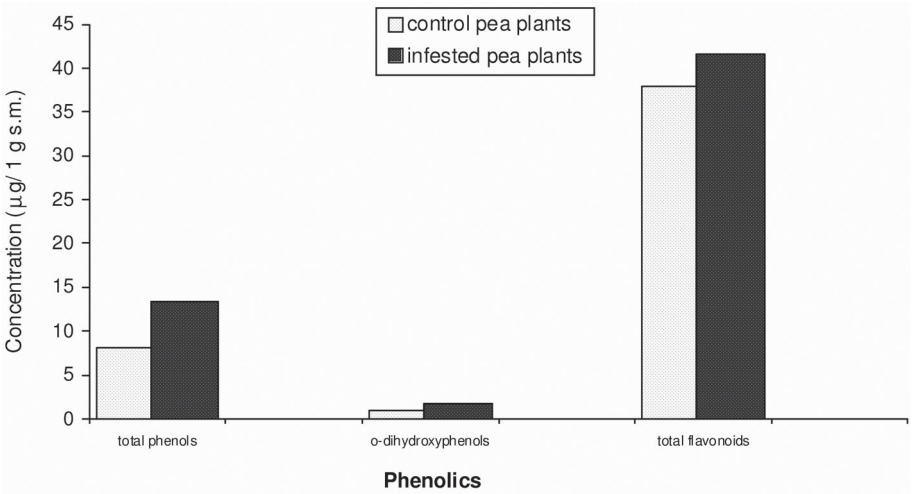

Figure 1. Concentration of phenolics from infested and control vegetative parts of pea plants.

Ferreres et al. [24] in the pea plants identificated quercetin and kaempferol derivatives. In our study both pea cultivars – control and infested plants had similar flavonoids profiles (Figure 2). On the basis of the absorption spectra of the chromatograms, six flavonol aglycones were identified: quercetin, kaempferol +RCO^-, kaempferol, tricin, apigenin+RCO^-, and apigenin. Statistical differences in the content of flavonoids in control and infested vegetative pea plants were not found (test G, G = 7.62, df = 5, p = 0.17). However, infested pea plants were characterized by high content of identified flavonoids, except kaempferol +RCO^-. In infested pea plants, flavonoids content ranged from 1.83 µg/ g d.w. (tricin) to 16.40 µg/g d.w (apigenin +RCO^-). In control pea plants the content of kaempferol were distinctly higher (15.54 µg/ g d.w.) in comparison to infested pea plants (10.08 µg/ g d.w.).

Among all identified compounds in infested and control plants, tricin content was lower than others and damaged 1.03 and 1.83 µg/g d.w. for control and infested pea plants respectively (Figure 3).

Studied chemicals are biologically active substances and could be used as pesticides or compounds affecting the behaviour of insects. Further studies on plant chemistry and insect-plant interactions are needed.

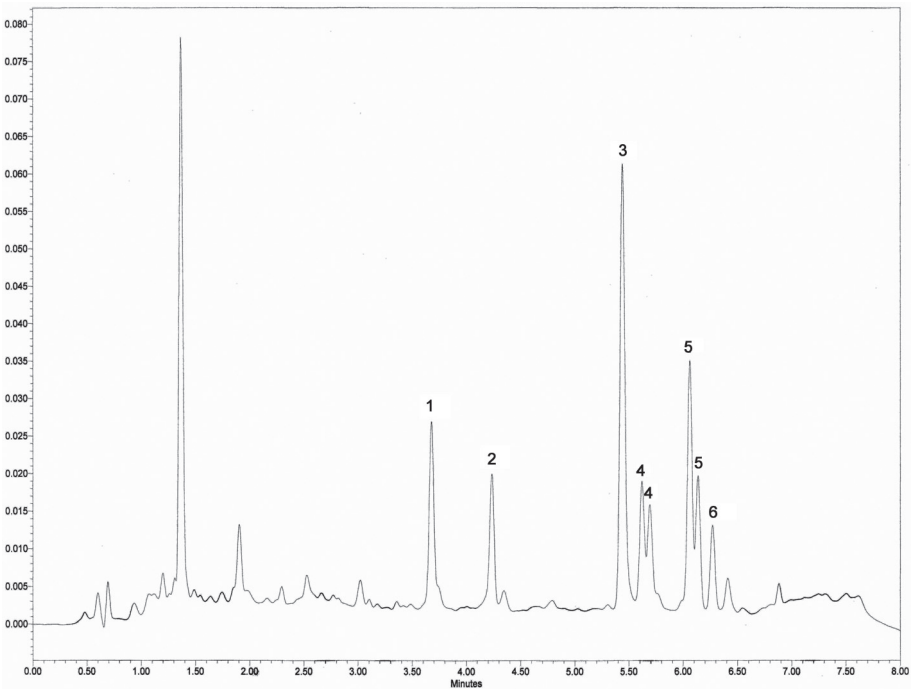

Figure 2. UPLC profile of flavonoids from vegetative parts *Pisum sativum*. 1- quercetin, 2 – kaempferol, 3 kaempferol+RCO^{-}, 4 – tricin, 5 – apigenin+RCO^{-}, 6 – apigenin.

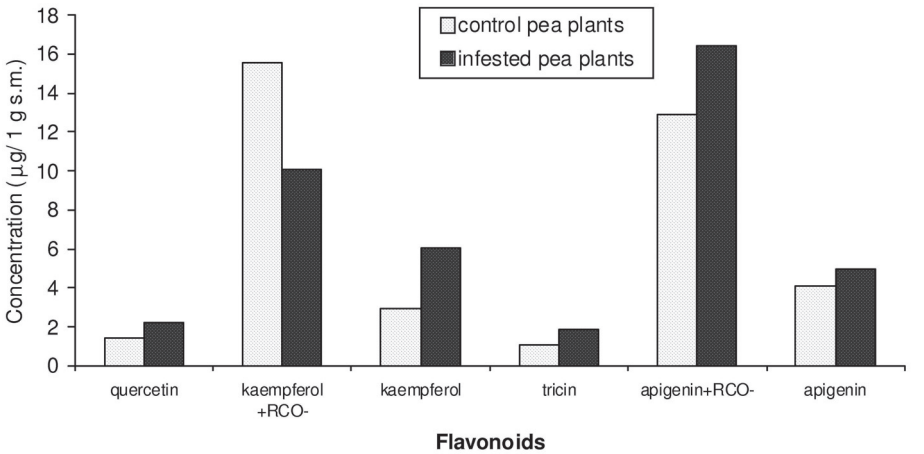

Figure 3. Concentration of measured flavonoids from infested and control vegetative parts of pea plants.

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